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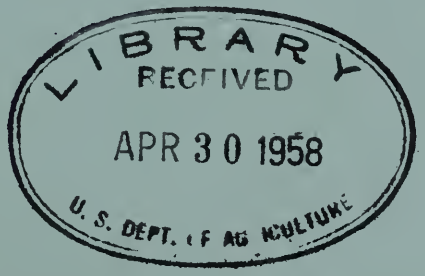
PROCEEDINGS of Symposium on Vesicular Diseases

PLUM ISLAND

ANIMAL DISEASE LABORATORY

September 27-28, 1956



U.S. Agricultural Research Service

UNITED STATES DEPARTMENT OF AGRICULTURE

During the week of September 24-28, 1956, at Plum Island, N. Y., a new laboratory for research on foreign diseases that constitute a threat to this country's livestock was dedicated by the United States Department of Agriculture.

At this new laboratory scientists will study characteristics of various types and strains of viruses--including the foot-and-mouth disease virus--how they spread, methods of artificial propagation, diagnostic procedures, disinfection methods, and preventive measures. The laboratory also provides facilities for identification of the causative agents in disease outbreaks where foreign diseases are suspected.

The scientific highlight of the dedication activities for the Plum Island Animal Disease Laboratory was a 2-day symposium on foreign animal diseases, particularly foot-and-mouth disease. Participating in these discussions on September 27-28 were prominent scientists and research workers from several foreign countries as well as the United States. Papers presented at this symposium are included in this booklet.

Issued December 1957

PROCEEDINGS OF SYMPOSIUM ON VESICULAR DISEASES

Plum Island Animal Disease Laboratory

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TABLE OF CONTENTS

*Since the opening of the Plum Island Disease Laboratory, a realignment within the Agricultural Research Service has resulted in the change of Branches to Divisions.

TABLE OF CONTENTS--Continued

PROGRESS IN ANIMAL DISEASE RESEARCH

B. T. Simms[1/]

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Mr. Secretary, Dr. Shaw, Dr. Shahan, Honored Guests, Ladies and Gentlemen: please allow me to join Dr. Shahan in welcoming you to Plum Island. We appreciate this opportunity to visit with you, to show you our new laboratory and its facilities, and to tell you how we plan to use them.

I want to talk to you today about a very big business in which all of us are interested--production of livestock and poultry and their products; about why this business is big; about why we want it to become even bigger; and about some help it will need if it is to grow.

How big is this business? Our farmers and ranchers receive over 15 billion dollars each year from the sale of farm produce of livestock and poultry and their products. Can you visualize a 15- to 19-billion-dollar operation? If you are like I am, it is hard to do. Some comparison may help. This amount of money is about twice the total wholesale price of all the passenger automobiles that are being made in our country this year. Or, it is more than eight times the cost of all air and rail passenger transportation in our country during 1955. Yes, this is indeed a big business.

Why is it so big? It is big for two reasons: First, our 167 million people want it to be big. They want meat, milk, butter, cheese, and eggs in their daily diets; wool and mohair for their fabrics; leather and hides for a thousand uses; and insulin, adrenalin, and other medicinals of animal origin for treating certain diseases.

Second, we have the ability to produce these products that our people want. Our farmers and ranchers produce about one-fourth of the world's total supply of red meat, although we have less than a fifteenth of the total world population. The meat, the milk, butter, and cheese, and the eggs we eat are both good for us and good to us. They bring us many of the vitamins, minerals, and high-quality proteins that we need. And they add zest to our eating and pleasure to our living.

But we mustn't become complacent. If we continue production of foods of animal origin at only the present level, our ever-increasing

1/ Retired.

population will have a scant supply in 5 years, a seriously short supply in 10 years, and a critically insufficient supply in 25 years. We must not let this happen. History has indelibly written that revolution, anarchy, and tyranny are fellow travelers of hunger and malnutrition. Our plans for the future must include an ever-abundant supply of these foods if we want our people to be strong and our nation to endure.

A brief look at the past may help us to plan the future. Fortunately, our forefathers found very few destructive diseases waiting to welcome their livestock when they came to this country. And the sailing ships they came on moved so slowly that animals suffering from most of the destructive communicable diseases when they went aboard either died or recovered before they sighted America. With almost no man-made transportation facilities at hand, the sick animals that did arrive usually didn't move far enough or frequently enough to spread disease very widely.

Furthermore, the small livestock population of early days made it difficult for transmissible diseases to spread rapidly. During colonial days and the first 50 to 60 years of our republic our country was relatively free from widespread destructive plagues and epizootics among our livestock. The best record I can find--and I know it isn't based on very good data--says we produced and ate well above 200 pounds of red meat per person per annum during the two decades just before the Civil War.

But with the coming of the steamboat and the steam locomotive and later the gas engine, long-distance movement of farm animals and their diseases became commonplace. Europe was separated from us by months in the middle of the 18th century, by weeks a hundred years later, and now less than a day away still another hundred years later. It took 12 years for hog cholera to spread to 10 States after it was first recognized in Ohio about 125 years ago. But in the next 30 years, as railroads were built, hog cholera became the master killer of swine in almost every community in which hog production was an important industry. Southern cattle that were moved by boat up the Mississippi River carried ticks, tick fever, and death to thousands of cattle along the upper reaches of that river and its tributaries. Glanders in horses, tuberculosis and brucellosis in cattle, fowlpox, sheep scab, cattle scab, and worm parasites of cattle, sheep, and swine became increasingly widespread and destructive as our transportation facilities increased and improved.

At least partly because of the ravages of diseases and parasites our meat production per capita decreased gradually from about the middle of the last century until about 20 years ago. Our exports, first of live animals and later of meat, fell off rapidly in the early years of this century and became relatively insignificant by the end of World War I. In spite of this, meat consumption per capita continued to drop.

Please don't think we were doing nothing about these diseases during all this time. More than 150 years ago North Carolina tried to prevent spread of tick fever by establishing quarantines. Massachusetts eradicated pleuropneumonia from its cattle nearly 100 years ago. Quarantine regulations to prevent introduction of foreign diseases were in effect far back in the last century. Research to find methods of controlling, eradicating, and preventing transmissible diseases and parasites of farm animals was begun in the Department of Agriculture more than 70 years ago. Gradually, as this and other research gave us better tools to work with, we began winning the war against some of these scourges. Tick fever has been eradicated. Tuberculosis and brucellosis in cattle, sheep scab, cattle scab, pullorum disease, fowlpox, and hog cholera have been brought under fairly good control. Meat production per capita has increased rather steadily during the last 20 years.

As you know, our future plans include a new animal disease laboratory at Ames, Iowa. It will enable us to expand our research with the diseases already with us. We believe with the knowledge already at hand and that which will come from Ames and the other laboratories in this country, we can hold our own against most of the diseases already present in our country. We have high hopes that research will bring us techniques and procedures that can be used to eradicate some of the diseases that are only controlled at present.

We are, and have been, for a good many years very much concerned over the possible introduction of foreign scourges such as foot-and-mouth disease, rinderpest, African swine fever, Rift Valley fever, Teschen's disease, fowl plague, and surra, to mention only a few. We know the threat of these diseases becomes more real with every improvement of the airplane and every extension of our world trade. Not having been exposed to them our present livestock and poultry population is very susceptible to these foreign diseases. Any one of the more destructive group could bring disaster if it broke through our quarantines and became well established. Here at Plum Island we shall try to find better methods of keeping these diseases out and of coping with them if they get in. We are already studying foot-and-mouth disease here. That work will be greatly expanded when we occupy this new building. We shall continue to coordinate our work with that under way in other foot-and-mouth disease research laboratories throughout the world. We hope and expect to study others of these foreign diseases in the future.

This laboratory, the new one we are to build at Ames, Iowa, the different State agricultural experiment station laboratories, the endowed veterinary research institutions, and veterinary research laboratories in other countries of the world will all stand in the future as guardians of this big business in which we are all so vitally interested. Of course, we don't expect to solve all the

disease problems. But, we believe we--and by we I mean the research workers both in our country and abroad--can find enough answers to enable livestock and poultry production to continue to expand.

We are enthusiastic and excited over this new laboratory. Our staff is looking forward eagerly to the time when it will be in full operation. We know we are facing days and weeks and years of hard work, but it will be work that we love. We can expect many disappointments, but we can expect them to be overshadowed by achievements.

The Department's former research workers with animal diseases have passed on to us a great heritage of achievement. Our promise to you, Mr. Secretary, to the millions of producers of farm animals and their products, and to our entire population that is dependent on these products is that we shall make every effort to merit this heritage.

Thank you.

DEVELOPMENTS IN VIROLOGY

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The word "virus" is a very old term used long before anything was known about the nature of any agents that produce infectious diseases. It was a general term referring to the contagium of such diseases; to the vague something which transferred these diseases from one individual to another.

When Iwanowsky (14),[1/] in 1892, found that filtered extracts of diseased plants containing no bacteria or other recognizable cells would produce mosaic disease when placed on susceptible plants, the term "filterable virus" was coined. A considerable number of filterable viruses were recognized within a decade, several hundred are known today, and others are being found with considerable frequency. Today, we know that many of the more serious infectious diseases of man, animals, plants, and even bacteria are caused by agents that belong in this category. In recent years it has become evident that the original criterion for identifying these agents, that is, filterability, is not so important as it was once believed, for it is known that many viruses are made up of elements that are so large as not to be readily filterable. The prefix "filterable" is now generally dropped and these agents are known under the term "viruses"--the word that now has a specific meaning.

Although the first virus was recognized more than 60 years ago, very little was learned about the nature of these agents for almost 40 years. Until about 1930 very little was known about viruses, except that they would produce diseases in living hosts, that they would, in most instances, pass filters impervious to agents as large as bacteria, and that they could not be cultivated on any of the media commonly employed for bacteria. Many of the old textbooks on microbiology headed their chapters on virus diseases by such titles as "Diseases of Unknown Etiology--Filterable Viruses". In general, the viruses were recognized by their effects--by their ability to produce characteristic disease changes in plants and animals--rather than by any qualities of their own. Pasteur believed that his failure to detect the agent of rabies in infective nerve tissues was because it was too small to be seen with his microscope. Most of the early workers had the same concept of the nature of viruses--that they were cellular organisms like bacteria, except much smaller.

1/ Figures in parentheses refer to "References", at the end of this article.

There was much speculation about the nature of viruses but little experimentation during the long period when there was essentially no progress in the understanding of them. This period we might call the dark ages of virology if we overlook the fact that at that time there were not enough evidences upon which to base the name for a new field of science. The physical and chemical techniques that have yielded so much information about viruses during the past 25 years had not as yet been developed. The list of virus diseases grew mainly because bacteriologists, having exhausted their efforts and techniques in unsuccessful efforts to find disease-producing bacteria in many diseases that were obviously infectious, used the virus category as a garbage pail into which they tossed all their unresolved residues. Many diseases once thought to be of viral origin are now known to be caused by other agents; many others once thought to be caused by other agents are now known to be caused by viruses.

Beginning about 1930, a remarkable series of new techniques were developed. Because of these new working tools, we owe the considerable increase but still imperfect knowledge of the viruses we now have. I shall mention, in the order in which they appeared, the techniques that I consider most important.

Modern Techniques of Virology

1. Demonstration of Virus Activity in Inclusion Bodies

Woodruff and Goodpasture (28), in 1929, showed that the intracellular bodies found in fowlpox, which had been named for Bollenger, could be separated from the host cells, and that these separated bodies carried the pox virus. In 1930 it was shown that these relatively large bodies could be broken down by tryptic digestion releasing many minute spherical elements which were resistant to this enzyme and which possessed virus activity. This was the beginning of the unravelling of the mystery of these bodies, a demonstration that they were neither specific degeneration products of the cell cytoplasm, nor protozoa or other organisms, but really colonies of virus elements.

2. The Chick-Embryo Method of Culturing Viruses

Woodruff and Goodpasture (29), in 1931, introduced the chick-embryo technique for studying and propagating many viruses. The original workers used the method for cultivating a virus that was pathogenic for chickens. Later it was found that many viruses which had no pathogenicity for chickens could be propagated in chick embryos.

3. Graded Collodion Membranes for Determining Particle Size of Viruses

Elford (5), in 1931, published his first paper on the use of collodion membranes of graded porosity for determining the approximate

particle size of viruses in suspension. With this technique the size of the particles of many viruses was determined. It was found that not all viruses were as minute as had been thought; that they varied from ones that were exceedingly small, such as that of foot-and-mouth disease that has a diameter of about 10 millimicrons, to others that were more than 40 times as great in diameter such as the virus of psittacosis.

4. The Chemistry of Viruses

The chemical study of viruses has been handicapped by two circumstances: (1) In attempts to purify viruses, many of them are easily inactivated by chemical manipulation and are lost; (2) It has been difficult to identify and separate viruses from the cellular fluids and debris, with which they are always associated.

The first noteworthy chemical triumph in the field of virology was made by Stanley (25), in 1935, when he isolated the virus of mosaic disease of the tobacco plant. This was found to be a crystalline nucleoprotein of extremely high molecular weight. Subsequent studies have shown that this virus is far more resistant to chemical manipulation, and it appears to be chemically simpler than most other viruses. A number of other plant viruses have been obtained in crystalline form, however, but for 20 years none of those affecting animals were successfully isolated by crystallization. This led to a strong opinion among virologists that plant viruses were quite different from those which affected animals. In 1955, Schaffer and Schwerdt (21) announced their success in obtaining a strain of poliomyelitis virus in crystalline form. If this is true, then the idea of the gulf between viruses of plants and animals may have to be revised.

5. The Development of Ultracentrifuges

Since most viruses did not lend themselves to chemical purification, because of their fragility, physical means of accomplishing this purpose were adopted. The principal means of effecting such separation and purification is differential centrifugation. Some of the very early workers had concentrated some of the larger viruses with the centrifuge but most of them required far more power or speed than was offered by the standard laboratory instruments. An air-driven ultracentrifuge was introduced into the study of virology by Bauer and Pickels (1) in 1936. Improved and less expensive equipment has been introduced in recent years. These instruments capable of speeds of 50,000 r.p.m. and more, and of creating centrifugal fields of from 50,000 to 100,000 times gravity are able to sediment the smallest of the known viruses.

6. Tissue Culture Techniques

Viable tissue cells, suspended in fluids *in vitro*, were used to propagate certain viruses as early as 1925 (Parker and Nye, 15), but

little practical use was made of the method for many years. In 1933, Gey (9) introduced a method of cultivating animal cells in a plasma clot in which the clot is alternately bathed in a nutrient fluid and in air--a technique which has come to be called the roller-tube method. In 1939, Gey and Bang (10) reported their success in maintaining the virus of lymphogranuloma venereum for more than 9 months in such a cell culture. The method was used by Feller, Enders, and Weller (7) in 1940 to produce vaccine virus, and by a few others to cultivate other viruses. It was not until Robbins, Enders, and Weller (16), in 1950, reported success in cultivating the virus of poliomyelitis in roller-tube cultures that the method became popular with virologists. This technique has proved to be very useful in recent years for many purposes and has been the means of discovering a series of new viruses that at present could have been detected in no other way.

7. The Electron Microscope

The electron microscope, which makes it possible to resolve objects infinitely more minute than can be seen in ordinary microscopes, came into use in the field of virology about 1945. The early electron micrographs were rather disappointing in their lack of detail but in more recent years their quality has improved tremendously. The use of metallic shadowing has contributed much to the improvement. Visual confirmation of dimensions that previously had been known only by calculations from centrifuge and filtration data were thus obtained. It was further demonstrated by these micrographs that all virus particles were not more or less spherical as had been previously supposed, but there were many shapes, such as cuboidal, rod-shaped, and tadpole-shaped. Electron micrographs of chemically treated viruses have provided visual evidence of their structure in some instances, confirming data that had been obtained by chemical investigation.

8. The Use of Antibiotics in Virus Isolations

Since the majority of viral agents are not susceptible to the action of antibiotics, the use of antibiotics has simplified the process of isolating, identifying, and cultivating these agents when they are in the presence of bacteria which often overgrow and obscure them. Antibiotics have made it possible, in fact, to isolate viruses with relative ease from such heavily contaminated materials as feces and bronchial exudates.

These are not all of the newer techniques by any means, but it seems to me that they have been the ones that have had the greatest impact on the newly developing science. The use of suckling animals as experimental subjects, the use of cortisone and irradiated animals for the same purpose, the alternating host to host technique for adapting viruses to unnatural hosts, the newer serological methods, including the hemagglutin and hemagglutinin-inhibition tests, are other newer methods that have been useful. Although these methods have answered

many questions on the nature and mode of operation of the viruses, many remain to be answered. Suggestions for answering some of these questions may be found in current work. I shall review some of the trends, interests, and discoveries in the field of virology.

Viruses and their Relation to Malignant Tumors

Cancer is of increasing concern to mankind since many more people are now living in the "cancer age". Since Ellermann and Bang (6), in 1908, showed that a malignant blood dyscrasia of fowls (leukemia) could be transmitted to normal birds with cell-free filtrates, there has been much interest in the possibility that many malignant tumors may be the result of viral activity. Interest in this subject was quickened by the work of Rous (17), in 1910, which brought to light a solid tumor, a sarcoma, which could be transmitted in the same way. Several dozen virus-induced tumors have been described in later years. Many of these are laboratory curiosities or, at least, are not of great importance in nature. They serve to keep alive the thought, however, that viruses may be the underlying or contributing cause of the deadly cancers that destroy so many people and a considerable number of animals.

Shope (22) demonstrated that his rabbit papilloma virus was able to persist in an altered or masked form in the tumors induced in domesticated rabbits. Since these tumors frequently become cancers after some months (Rous and Beard, 18), there are many who feel that such a virus mechanism may be operative in many or all carcinomas. Shope (23) in his report on his recent visit to Soviet Russia says that a number of Russian laboratories are working on this hypothesis.

The Oncolytic Viruses

Of considerable interest in a number of laboratories at the present time is the possibility of finding viruses, or producing variants of known viruses, that may be used to specifically antagonize the cells of cancer. Eagle and coworkers (4) reported that the cells of an epidermoid carcinoma of man, grown in tissue culture, are specifically lyzed by the viruses of poliomyelitis, herpes simplex, vaccinia, and a number of adenoviruses. Others have tested many other viruses on cancer cells grown in tissue culture, and also on natural and experimentally produced carcinomas in living animals. Viruses have even been used experimentally on human patients who were about to die of cancer. I am not aware of any claims that carcinomas in living animals have been utterly and completely destroyed by virus injections, but massive lysis of cancer tissue has been brought about in both man and animals, and the progress of such tumors in animals has been greatly retarded. The hope of the experimenters is, of course, that viral agents may be first adapted in tissue culture to become strongly cytolytic to cancer cells and that the adaptation may be so perfect as to make it possible, by injections of the adapted virus, to destroy

the cancerous cells without seriously damaging the closely related cells of the host's body. Certainly this search will be carried on vigorously, and it doesn't seem too sanguine to hope that eventually a specific form of cancer therapy may be developed.

Immunity to Viruses

It has been assumed that the principles of immunity, which were worked out largely with bacteria, also apply to viruses. Viruses contain antigens. Consequently, when they are injected into animals, antibodies are produced. Agglutinating, precipitating, and complement-fixing antibodies may be produced with viral antigens as well as what are known as virus-neutralizing antibodies. The latter, when mixed with virus in proper proportions, *in vitro*, prevents it from exerting its disease-producing properties when the mixture is injected into susceptible animals. It is known in many cases that viruses are not destroyed by these antibodies because it is possible to restore virus activity by centrifuging the virus particles out of suspension and thus separating them from the restraining influence of the antibodies.

A rather quiet controversy has been going on for some years between the advocates of inactivated-virus vaccines and live-virus (attenuated) vaccines. The advocates of the inactivated-virus vaccines feel that these so-called "killed" virus vaccines are capable of giving useful immunities and are safer than the live-virus products as living viruses possess the potential of acquiring enhanced virulence and thus may be dangerous. The advocates of the active "live" virus vaccines, in some cases, doubt that an inactivated virus is capable of immunizing. In any case they claim that live-virus vaccines generally will confer a stronger and more lasting immunity. They also believe that any immunity conferred by a so-called "killed" virus vaccine is due to the presence in the product of active virus that has escaped inactivation by whatever process has been used to accomplish this end. They argue that the inactivating process may only serve to "mask" virulent virus and that such vaccines may not be as safe as frankly live-virus products in which any active virus is attenuated.

This controversy was fanned into activity when the accidents with some lots of the Salk vaccine for poliomyelitis became known in 1954. The Salk vaccine is inactivated, but it was proved afterwards that the lots of vaccine which caused trouble contained active virus. The one group pointed to this as proof of their contention that inactivated-virus vaccines are not always safe; the other attributed the accidents to faulty preparation and inadequate testing.

Sutton and Brooke (27) and Smith, Mamay, Marshall, and Wagner (24) have described a similar experience with a supposedly inactivated vaccine made to protect laboratory personnel against the Venezuelan type of equine encephalomyelitis. This vaccine produced active infections in at least 14 persons of a group of 327 that received it.

Testing of the accused lots of vaccine, both before and after it had been used on man, failed to demonstrate any viable virus in them. No satisfactory explanation of the phenomenon has been made, unless, perhaps, man is found to be more susceptible than any of the laboratory animals used for testing the product and thus became infected with doses which were subinfective for the test animals. In the literature there are a few reports of paralytic syndromes following the use of supposedly inactivated rabies virus vaccines in which active virus was isolated from the victims. These generally have been explained on the basis of errors in production methods and safety tests. The question has been raised, however, as to whether we can exclude the possibility that supposedly inactivated vaccines may be reactivated in the tissues when the processes of absorption have removed the inactivating or masking substances.

Those who conceive of viruses as biological entities, or living cells, will naturally think of the processes of immunity in virus diseases as being identical with those with which we are more familiar in bacteria-induced infections. They will argue that there is every reason for believing, both from analogy and experience, that the pathogenic properties of viruses can be destroyed by chemical or physical means without destroying or inactivating their antigenic or immunizing properties, and they see no reason to doubt that fully inactivated viruses can induce serviceable immunity.

There are some reasons that should warn us that antiviral immunity may not be wholly analogous with antibacterial immunity. For the most part, bacteria multiply in the fluids of the body, whereas viruses multiply inside of cells. The "blocking" or interference phenomenon is an immunological phenomenon seen in virus infections but not in those due to bacteria or other infective agents. Antibodies apparently have nothing to do with this reaction since the response occurs too rapidly. The mechanism by which the susceptible cells of a particular host are protected from a virus by some sort of barrier established by another virus infection which immediately preceded it is not understood. Some virus infections appear to produce an absolute immunity, which is lifelong with persisting antibody titer rather than a relative immunity, as is observed in antibacterial immunities that are usually shorter lived and fade rapidly after convalescence.

The entire matter revolves on the question of whether or not our present techniques are adequate to fully inactivate virus vaccines without denaturizing and thus destroying the immunogenic materials in viruses and whether viable viruses exist in amounts or conditions in the so-called inactivated-virus vaccines which make their detection by present methods uncertain or impossible. These can be settled only by future research.

The Orphan Viruses

The possibility that a large number of viruses might exist in nature unrecognized because they did not produce recognizable changes in plants and animals has long been a subject for speculation. Until recently there was no way to detect such agents. Tissue culture methods, especially the roller-tube technique, have made it possible to find and isolate viruses from such highly contaminated materials as feces. In using the method to search for the virus of poliomyelitis in the stools of children in nonepidemic regions and time periods, not only have a surprising number of poliomyelitis virus isolations been made but a number of other viruses, not known to be connected in any way with disease in the host, have been found (Sabin, 20). These agents have been found only because they cause lysis of cells in the cultures. Since it is known that many disease-producing viruses do not lyze cells in cultures, it is probable that the new agents represent only a fraction of those that exist--the others being undetectable by present methods.

These viruses, which have no known connection with any disease process, have been called orphan viruses. Presumably some of them eventually may be found to have some association with disease, but it may well be that there are many viruses in nature which are low-grade parasites producing so little damage to their hosts that we are unable to detect them.

Orphan viruses have been found in monkeys and undoubtedly will be found in many other animals when they are searched for. The virus (Miyagawanella bovis), which York and Baker (30) isolated from the intestinal content of calves and which seems to be widely distributed in cattle might be considered as a member of this group.

The Adenoviruses

Of great interest today are the new viruses that have been isolated from the upper respiratory tract of man. These new viruses appear to be involved in the febrile, upper respiratory diseases that play such an important role in human health. Huebner and coworkers (13) called these agents the Adenoidal-Pharyngeal-Conjunctival (A P C) viruses. Hilleman and other coworkers (12) called them the Respiratory Infection (R I) viruses. By agreement both of these terms are now being dropped in favor of a new one--the adenoviruses.

The adenoviruses have common complement-fixing properties, yet they fall into at least 14 serotypes (Rowe, et al., 19), 12 of which were isolated from man and 2 from monkeys. All grow readily in cultures of HeLa cells, and they signal their presence by producing cytolysis. None of them have shown any pathogenicity for experimental animals, but there is accumulating evidence that some of these types, at least,

are concerned with acute symptoms of the host. Bell and coworkers (2) have shown by studies on naval recruits and Hilleman and coworkers (11) on army recruits that vaccines made from certain of these viruses greatly reduced respiratory infections during the winter months.

I am not aware of any attempts to find viruses of this type in animals other than the monkey, but one can hardly doubt that they exist. In view of the fact that we have a number of respiratory diseases of major importance in domestic livestock in which the etiology is still clothed in considerable uncertainty, it would appear that this would be a worthwhile field for investigation.

The Chemical Structure and Nature of Viruses

Great progress has been made in recent years in acquiring a better understanding of the nature of viruses through studies made of highly purified agents. Much of this work has been done with plant viruses that have been readily crystallized and thus freed of extraneous substances derived from the infected host cells. Most of the work with animal viruses has had to be done with viruses purified by centrifugation. Obviously this method is less efficient in removing all contaminating materials, since host materials that have physical properties similar to those of virus will be separated with the virus fraction.

All viruses appear to be made up of nucleic acid and a protein; some have lipids and carbohydrates as well. The nucleic acid, which is similar to that found in the chromosomes of higher plants and animals, is believed to be the bearer of the genetic principle of the virus, that is, the portion of the virus molecule which is concerned with multiplication or replication. The protein portion is believed to be the fraction that determines immunologic specificity; that is, it is the portion which carries the specific antigens. There is evidence that the nucleic acid portion forms a sort of core which is surrounded by the protein.

Fraenkel-Conrat and Williams (8) recently claimed to have produced a sort of synthetic virus by recombination of virus fragments. By chemical manipulation the nucleic acid and the protein portions of the crystallized mosaic disease virus were separated. Neither fraction possessed virus activity. When the fractions were recombined there was no immediate virus activity, but after about 1 hour the typical activity appeared. Similar observations have been made with some of the bacteriophages.

One of the latest conceptions of virus structure and formation has been given by Burnet (3, p. 1101) who, speaking about human influenza, describes the process of cell infection as follows:

"1) The virus particle attaches itself to the free surface of the cell--the union being mediated by an attachment of an enzyme-like component of the virus surface to prosthetic groupings (recently defined by Gottschalk, 2,) of cell-surface mucoprotein.

"2) The virus particle enters the substance of the cell and rapidly loses its existence as an infective particle in the sense that an extract of that cell is no longer infective for a fresh embryo.

"3) About 3 hours after infection, new virus is detected in the cell; and from about four hours onward, new infective virus is being liberated from the cell. There is good evidence to suggest that the production of new infective virus takes place at the free surface of the cell."

This conception differs from the situation that exists when a bacterium or other parasite has entered and multiplied within a cell. In the latter the multiplying organisms may eventually consume the entire content of the cell and thus destroy it, but at every stage of the process the parasite can be recognized as a separate entity within the cell. The virus concept is different in that when the cell has been invaded its identity is lost since its substance is apparently broken down to components that unite with others provided by the host cell. From this fusion of materials a new virus is produced. Virus is thus composed partly of substances supplied by the host cell. As Burnet (3, p. 1102) stated, "There is much to suggest . . . . . that a considerable proportion of the material contained in what is conventionally regarded as purified virus particles is very directly derived from the host cell." Stanley (26) claims that the crystallizable plant viruses contain no substances that can be identified with the host cell; however, there are a number of animal viruses that, when purified as fully as present methods will allow, show immunologic relations to the host species in which it was produced. It is impossible to know definitely whether these are real components of the virus or tissue contaminants that have not been removed. Some highly competent virologists evidently do not believe that they are contaminants.

The traditional biologist, accustomed to thinking of the cell as the ultimate unit of living matter, has much difficulty in comprehending some of the new concepts about viruses. The old arguments, whether viruses are living agents or nonliving autocatalytic chemical agents, seem to have largely subsided; yet, it does not seem to me that the question has been settled. Possibly we are less sure today than yesterday as to what constitutes life. The electron microscope has served to reassure those who visualize viruses as bits of living matter by providing proof that such bits actually exist. Crystallization of viruses, however, and particularly the degradation and recombination experiments make it difficult to conceive of the end product as anything endowed with life.

Whatever their nature, it has become clear during the last quarter century that we are just beginning to learn something of a group of agents that have a profound influence on human health and welfare. Virchow's cellular pathology has served us well for nearly a century, but pathologists of the future apparently are going to have to look beyond the cell to perceive some of nature's important secrets.

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PREVENTIVE VACCINATION AGAINST FOOT-AND-MOUTH DISEASE

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In a number of countries only sanitary measures are used for combating foot-and-mouth disease. Among these slaughtering of all susceptible animals that have been exposed to the infection--so-called stamping-out--takes an important place.

For economical reasons application of such radical steps, however, is only possible in regions that are not too heavily infected. As a rule, they are only employed for the eradication of the disease from newly invaded territories that have previously been free, and in which it has not yet spread widely.

Stamping out has been further used for the cleaning up of the last foci of infection at the dying out of an epizootic.

In regions not protected by water or mountain ridges, that may be exposed more or less continuously to massive infection from neighbouring areas, sanitary measures have very often not yielded the results expected from them. Under such circumstances the most thorough eradication measures might well involve a large percentage of livestock, and on that account be impracticable. It is then that vaccination has been used with most success to cut down losses, that otherwise might have been very extensive.

In some regions of Asia religion forbids the native populations to kill the cattle; in others, nomad tribes have to keep cattle moving about because of the lay of the pasture grounds. In such cases, a stamping-out policy, as well as most other sanitary measures, is bound to fail. Indigenous cattle are not very susceptible to the virus of foot-and-mouth disease, and vaccination is the only possible means of protecting the relatively small numbers of animals of foreign breeds present.

Some differences of opinion as to the practical value of vaccination still exist. In recent years a number of reports on its effect on the development of different epizootics has been published.

Many workers, who previously have witnessed the failure of sanitary measures to prevent the spread of the infection, are highly impressed by the results obtained by means of early vaccination, especially if used on a large scale. Both RÖHRER and UBERTINI, who studied the effects of the treatment in the Eastern zone of Germany and in Italy, respectively, stressed its value. A number of unsatisfactory

results has been due to the fact that vaccination has been often applied on too small a scale and too late. This for instance was the case in Belgium in 1951, according to WILLEMS.

The developments in Mexico, as well as the outcome of the A5 epizootic in Europe, have shown, that vaccination may be an effective tool, to keep the disease in bounds.

At the beginning of the A5 outbreak of 1951, no strain specific vaccine was available; however, in the Eastern zone of Germany, as well as in the Netherlands, cattle vaccinated against a related strain showed a very marked resistance. In both countries the numbers of infected animals and herds were low compared to those of neighbouring regions, where vaccination had been started later.

Recently, the influence of vaccination on the development of several foot-and-mouth disease epizootics in the Dutch province of Friesland has been investigated by DIJKSTRA. As this work probably is not generally known, it might be of interest to discuss it somewhat more extensively.

Friesland, which is one of our main cattle breeding districts, largely consists of a grazing area, covering its northern and western parts, and a more woody southeastern region. In this latter district the number of cattle per farm is much smaller than in the former. Foot-and-mouth disease epizootics in Friesland usually begin in summer or autumn. In winter, when cattle are kept confined, they develop much more slowly and show more of a tendency to come to a standstill.

Before vaccination was applied, the infection primarily spread through the closely stocked northern and western parts of the province, the eastern regions showing a much lower density of infection. The last cases in each epizootic were usually reported from these latter districts.

This pattern began to change in 1946, when limited quantities of vaccine first became available, which were largely used for the protection of the herds that were most endangered. A number of separate outbreaks now occurred, each of limited extent only, being kept in check by means of vaccination on the surrounding premises.

In the following years three smaller epizootics could also readily be brought under control by voluntary vaccinations, mostly carried out in the richer northern and western parts. Vaccination was much less employed in the other district. At the beginning of the 1951 outbreak a high percentage of the stock in the grazing area had been inoculated at least once.

The A5 epizootic approached Friesland from the southeastern side. A large number of cases was subsequently observed in the poorly vaccinated regions, but the virus did not succeed in establishing itself in the other parts of the province, where only a few isolated cases were observed. According to DIJKSTRA this result was entirely due to the use of vaccine.

In most countries only cattle are vaccinated routinely. From the development of the last epizootic in Germany RÖHRER concluded, however, that, depending on local conditions, it may be necessary to protect small ruminants too. For the successful prophylactic treatment of swine, special vaccines must be prepared, because the virus strains now in use do not give satisfactory results in this host.

In order to be most effective, vaccination should be used on the largest practicable scale and under continuous control of the veterinary authorities. The vaccination programs applied differ from country to country.

In Switzerland vaccination is routinely used on farms surrounding infected premises, all animals having been directly exposed being slaughtered.

The Belgian veterinary services are continuously vaccinating in a zone of a depth of some 20 kms. along the frontiers. Recently vaccine also had been made available for other parts of the country.

Vaccination was introduced in the Netherlands in 1946. In the first years it was only used on a voluntary basis. In 1952 stamping-out was again employed to eradicate the last cases of the A5 epizootic. This measure combined with the prohibition of transport of all unvaccinated cattle, has highly stimulated the use of vaccine, so that at present virtually all bovines of older than 2 months are being vaccinated once a year against the A, O, and C types.

Although vaccination against foot-and-mouth disease has been used widely, and a number of laboratories are continuously engaged in research aiming at the improvement of production and control of the vaccines, an impressive number of problems remains to be solved. Some of these I would like to discuss with you to some extent.

In most vaccines now in use in Europe and a number of countries in Latin America, the virus has been adsorbed to aluminum hydroxide and subsequently treated with a low concentration of formalin at a slightly elevated temperature.

Formalin was introduced for the inactivation of the virus with a view to its use as a vaccine, by VALLEE in France as well as by BURBURY in England, but the vaccines thus obtained were found to

give only a poor immunity. SCHMIDT in Denmark later used colloidal aluminum hydroxide for adsorption of the virus. The adsorbed agent lost some of its infectivity but the outcome of innocuity tests remained inconstant, even after incubation at a higher temperature for some time. Finally the combination of the two treatments by WALDMANN was found to solve the problem. Addition of 0.05 percent of formalin and incubation at 25°C for 48 hours of a virus suspension that previously had been adsorbed to the hydroxide at pH 9.0 produced a safe and effective vaccine.

As a source of virus WALDMANN employed tongue epithelium as well as blister contents from cattle that had been infected artificially by injection of a large quantity of virus suspension intradermolingually. A yield of 40 to 50 grams was obtained per animal after 24 hours. This quantity was found to vary with the breed and weight of the animal, as well as with the virus strain used, but was not influenced by age or sex.

The amount of infective virus injected was especially of importance if a slowly multiplying strain was employed. Addition of some 50 percent of swine-passaged virus to the inoculum was found to have a favorable effect on the amount of infective tissue harvested. According to German workers, the amout of virus produced in one animal is sufficient for the production of 150 to 300 doses of monovalent vaccine. Workers in Mexico obtained a somewhat smaller return of infective material per head of cattle, but a smaller quantity of infective tissue was used per dose and the number of doses of vaccine obtained per head was higher. As this method of virus production is expensive, a maximum yield per animal is very important.

Immune animals have been shown to be completely unsuitable. Titrations of the virus obtained from cattle have been carried out, but most laboratories using this method of virus production do not do them routinely, virus yield usually being estimated by weight.

In growth curve experiments different strains have been compared as to the time needed for reaching a maximum of infectivity. For type O strains this was found to be the case after 12 to 18 hours; for strains of the A type, a slightly longer period was needed, that is, approximately 24 hours. It proved to be possible to shorten this span by carrying out a series of fast transfers. The highest titers then occurred after 6 to 12 hours, by 24 hours a marked decrease had taken place. It proved to be advisable to check the rate of virus development of the production strains routinely.

The method employed by WALDMANN and coworkers for obtaining the virus has a number of serious disadvantages. The material

produced is expensive, the necessity to use completely susceptible cattle only, may be a severe drawback for countries using large scale vaccination, as the large numbers of animals needed either will have to be imported or a fairly large percentage of stock--according to RÖHRER as much as 10 percent--may have to remain unvaccinated.

Large facilities for isolation have to be maintained, these demand large investments. The large numbers of infected carcasses produced forms a potential danger for spreading the infection, if the meat is to be salvaged for human consumption.

A number of different methods has been investigated in order to obtain a cheaper way to produce the quantities of virus needed for mass vaccination. Among these the tissue culture technique, the cultivation of virus in unweaned mice or in newly born calves, and the virus production in embryonated eggs seem the most important. Only the technique for the propagation of foot-and-mouth disease virus in explanted bovine tongue epithelium, as developed by FRENKEL, has so far been found to be an improvement of the original method.

For virus production according to this method tongues are collected at a slaughterhouse and transported to the laboratory as soon as possible. On arrival they are cleaned by rinsing with lukewarm water and subsequently brushed with 70 percent alcohol. Partial sterilization is then obtained by irradiation of the surface with ultraviolet light. The superficial hornified layers are sliced off with a rotating knife and discarded. The deeper layers are then collected in a culture medium consisting of modified Baker's solution of pH 7.4 to 7.6. Originally the tissues thus collected were minced further with scissors. This treatment, however, has been found to be superfluous and is now omitted.

After addition of the seed virus the cultures are incubated for 20 hours at 37°C, and are continuously aerated and stirred. Bacterial multiplication is controlled by means of antibiotics. Aeration with a gas-mixture containing oxygen has been found to be essential. Pure oxygen and oxygen containing 3 to 5 percent of carbon dioxide gave comparable results, sterile air was somewhat inferior. The volumes of the gas led through the fluid may vary within wide limits without affecting virus multiplication significantly.

Large steel tanks used as culture vessels containing the tissue harvested from as many as 300 to 400 tongues are presently employed. They may be heated by means of a water jacket.

It is very important that for inoculation a seed virus of a good titer is used. FRENKEL and RIBELIN found the highest peak of infectivity in cultures between the sixteenth and the twenty-second

hour. If a tenfold smaller inoculum was used, the time of maximum infectivity occurred a few hours later.

Tongue tissue is routinely obtained from cattle that have been vaccinated repeatedly in the field. Such tissues have been found to give quite satisfactory results in vitro, although in vivo, they do not support virus multiplication sufficiently. Material may be kept at refrigerator temperature for some time after collection, without showing a marked decrease in virus yield.

A great advantage of this technique of virus production is its low cost. Compared to those of the method formerly used, the possibilities for the dissemination of infectious material are markedly reduced.

Several investigators have continued work on the culture of foot-and-mouth disease virus in vitro. A number of different media has been investigated. Variations containing normal cattle serum, serum ultrafiltrate, or a protein hydrolysate have been found suitable by some workers. WILLEMS AND LEUNEN routinely added a quantity of cattle passaged virus to their inoculum. In the Netherlands continuous passages in culture are mostly employed, although in work with some virus strains an occasional passage through susceptible cattle has proved to be of advantage.

Contrary to what is usually done in laboratories utilizing virus obtained directly from cattle, virus harvested from cultures and used for vaccine production is routinely titrated by intralingual injection in susceptible animals.

A number of authors have compared the virus yields obtained in explanted tongue epithelium with those from artificially infected cattle. HENDERSON and GALLOWAY found no difference when comparing a cattle passage of an O strain from Venezuela with the 12th passage of the same strain in tissue culture. Titrations were carried out by intradermal injection into the tongue of susceptible cattle. They found a similar situation when studying two A strains, if the cultures were incubated for 18 to 24 hours, but met some difficulty when they tried to grow a third strain of this type in vitro. Occasionally this problem has been encountered by other workers. HENDERSON and GALLOWAY stressed that, the degree of infectivity of the culture tissue after a set period of incubation is closely associated with the virus content of the starting material. The same observation has been made many times in our laboratory.

FOGEDBY and JOHNSON, when working with a number of different strains, found infectivity, as measured by intralingual injection, to be higher

for cattle-passaged virus, if they compared the virus content per gram of infective tissue, with that of 1 ml of culture fluid. If their figures are recalculated, the amounts of infective virus produced per gram of tissue, however, are not significantly different.

A comparable result was obtained by UBERTINI et al., who studied an O2 and an A strain.

There is a possibility that, as in cattle, different virus strains multiply at a different rate when grown in vitro.

Opinions as to the suitability of infectivity as a measure for immunogenicity of a virus suspension, disagree. According to HENDERSON and GALLOWAY, and to GIRARD and MACKOWIAK, the protection obtained varies with the amount of antigen administered, and this quantity in its turn depends on the original virus content of the material used.

FOGEDBY and JOHNSON found, that long-term cultures showed a maximum antigenic value for guinea pigs after about 40 hours, although infectivity had markedly decreased by that time. MACKOWIAK observed, that cultures might vary widely in this respect, even if the same seed virus had been used. According to UBERTINI, a parallel exists between the amount of complement-fixing antigen formed in a culture and its immunogenic value, an opinion shared by FREDERIKS. HENDERSON found that in this respect strain differences exist. In some cases 40- to 60-hour-old cultures were superior to 24-hour-old ones, but this was not always so. This author is of opinion that only infectivity as measured at its maximum may be judged a fairly good measure for the antigenic value of older cultures. He found that the amount of complement-fixing antigen in cultures varied, together with infectivity.

FRENKEL and RIBELIN, who studied this problem with an O strain, observed that in older cultures complement-fixing activity sometimes increased, and at other times decreased after the peak of infectivity had been reached. Protective power of vaccines prepared from such cultures steadily increased when incubation was continued from 24 to 66 hours.

According to MOHLMANN, virus developing in vivo behaves similarly. In cattle a peak of infectivity occurs early, but vaccines prepared from such material are inferior to those produced from virus harvested later.

In some publications the immunogenicity of viruses grown in vitro and in vivo has been compared. In our opinion the most important of these is that by HENDERSON and GALLOWAY: These authors compared vaccines

containing equal amounts of antigen as calculated from the infective titer of the original suspensions, using a large number of cattle. The dose of each antigen protecting 50 percent of vaccinated animals was calculated. No significant differences were observed.

After doing a number of experiments of the same kind of guinea pigs, using a high passage culture strain employed in the field for vaccination of cattle, UBERTINI and coworkers concluded that the antigenic value of cattle-passaged virus was somewhat superior to that of the culture strain derived from it. The use of cultured virus for vaccine production, however, still appeared advisable.

It seems probable, that the more or less contradictory results obtained so far on the subject of the relative values of foot-and-mouth disease viruses grown in vivo and in vitro are, at least in part, due to our inadequate techniques for the evaluation of antigenicity.

For most of the vaccines used in the field, the principles developed by WALDMANN and coworkers in the late thirties have been retained, although some variations have been introduced.

Originally, the extract of 0.2 g of infective tissue was incorporated into one dose of 60 ml of a monovalent vaccine, which was to be injected subcutaneously. Half of this volume consisted of aluminum hydroxide gel containing 2% of dry Al_2O_3. The virus was adsorbed at pH 9.0 and inactivated by subsequent treatment with 0.05% formalin at 25°C for 48 hours. A pH of 9.0 was used, as the adsorptive capacity of the hydroxide was optimal at this value.

Innocuity tests were carried out by injecting 4 to 6 cattle subcutaneously with one dose of vaccine each, susceptible controls being kept together with the vaccinated animals. A challenge was performed 15 days later by swabbing the mouth with a cloth drenched in a suspension of the homologous virus. The vaccines were tried on a large number of animals under field conditions in several parts of Germany. Outbreaks that might have been due to an infective vaccine are stated not to have occurred. Resistance as observed in the field, was satisfactory. Immunity was thought to last more than 8 months.

Alterations introduced subsequently in the preparation of foot-and-mouth disease vaccines in some laboratories include the use of another pH value for adsorption and inactivation, as well as changes in the concentrations of aluminum hydroxide and virus. In Mexico and Denmark pH values of about 7.6 have been employed. Inactivation time probably should be prolonged in this case, but the problem does not seem to have been investigated thoroughly. The practical importance of changes of this kind is not known although longer incubation at increased temperature, as well as a higher formalin

concentration, might be expected to influence antigenicity unfavorably. On the other hand, a pH value of 7.6--according to PYL the virus' optimum--might be expected to counteract this tendency. The lack of a really satisfactory experimental animal makes itself felt here.

The reliability of an innocuity test depends, as HENDERSON pointed out, on the number of doses that can be tested. The introduction of the intradermolingual injection for the examination of foot-and-mouth disease vaccines may be an important improvement, at least for those virus strains that do not easily infect if administered subcutaneously. A disadvantage is that the amount that may be tested by intradermolingual injection per animal is rather small. Possibly the use of unweaned mice, as suggested by SKINNER, may be a further improvement. Some workers have a high opinion of the value of the guinea pig for this purpose.

Virus suspensions used for vaccine production are usually freed from contaminant microorganisms by filtration, although centrifugation and treatment with chloroform apparently have been also employed with success.

On examination by means of bacteriological culture media, vaccines should only occasionally yield some growth of nonpathogenic micro-organisms. Injection into experimental animals should further cause no harmful effects.

A number of points have been found to be of importance for the suitability of the aluminum hydroxide to be incorporated into vaccines. WUNDERLI and PYL have observed that the adsorption of congo red, which is usually employed for testing the adsorptive capacity of the gel, does not run parallel with that of the virus. The aluminum hydroxide used is mostly prepared commercially according to WILLSTÄTTER. The pH should be 7.0 or slightly higher; traces of ammonium sulfate seem to have a favorable effect on vaccine quality, although they do not influence virus adsorption.

Experiments carried out by FOGEDBY, in collaboration with MALMQUIST, OSTEEN, and JOHNSON, indicate that a much smaller quantity than that originally used may be sufficient for adsorption of the virus dose added. The application of more concentrated vaccines offers important economical and technical advantages.

In some countries of Latin America the intradermal-vaccination method developed by ROSENBUSCH et al., using a vaccine containing 4 to 5 percent of "virus" in 2 ml doses, has found general acceptance. According to WILLEMS, however, the immunity obtained in this way is slightly inferior to that developed after subcutaneous injection--the duration of protection especially being shortened.

The role of the aluminum hydroxide in the immunization process is not yet completely understood. Probably delayed liberation of the antigen is its major contribution.

According to a resolution passed by a committee of experts from the O.I.E. (Berne 1946), the aluminum hydroxide vaccine against foot-and-mouth disease should contain not less than 0.1 gm of infective tissue extract of each virus type per dose; this material should be infective for cattle in a dilution of at least 10^6. Vaccines should be bivalent, unless general conditions make another composition desirable.

The use of weight of infective tissue as a base for vaccine production complicates comparison between strains as differences in rates of multiplication and quantities of antigenic material produced do exist. Opinions differ as to the value of the complement-fixation test for this purpose, as mentioned earlier.

The use of infectivity for the evaluation of antigenicity, as is presently done in our laboratory and advocated by HENDERSON, has the drawback that a fairly large error is innate to the titration techniques now available.

Most authors agree that the dose of virus incorporated into the vaccine is of great importance for the level of immunity obtained, as well as for its duration.

A further point of consequence is the choice of the virus strain to be used. Preferably for combating an outbreak the homologous strain should be employed. The observation, however, that a solid immunity against one strain is, as a rule, sufficient for protection against field exposure to related strains, allows a wider use of vaccination than would otherwise be feasible.

Vaccines are usually tested by challenging vaccinated cattle after 2 to 3 weeks. As exposure by contact does not give satisfactory results, 2 other methods are currently employed. Either a known number of ID_{50} is injected intracutaneously into the tongue, or the tongue is swabbed with a cloth drenched in a virus suspension. Both techniques are rather severe as compared to the infection as it takes place under field conditions. The first method offers the advantage that a fixed degree of exposure is ascertained.

As a rule 4 to 6 cattle are used for every batch of vaccine. The evaluation of the outcome of such tests varies somewhat from laboratory to laboratory, but generally absence of generalization at the end of a certain observation period is accepted as proof of adequate protection.

Evidence as to the duration of the immunity obtained by vaccines from different sources is scarce--estimates varying from 4 months to almost a year. According to WILLEMS and LEUNEN, on the average, protection in the field lasts one year.

On the whole, there seems to be a parallel between the degree of protection as found 2 to 3 weeks after vaccination, and its duration.

Opinions as to the time the aluminum hydroxide vaccines keep their protective properties differ. On the one hand, WILLEMS has found that vaccines prepared in Belgium were completely effective after as much as 4 years. On the other hand, MICHELSEN has occasionally observed that vaccines that had been kept for 12 to 18 months were no longer good antigens and contained active virus. It is possible that differences in technical details of vaccine preparation play a role here.

A serious disadvantage of the present system is that development of immunity of individual animals cannot be studied.

Serological techniques for the evaluation of the immune response obtained by vaccination against foot-and-mouth disease in cattle have not been used on a large scale.

Recently we have been able to investigate the response of a number of cattle to type O vaccines by means of a neutralization test.

Blood samples were obtained a fortnight after vaccination, shortly before exposure. A good correlation was observed between clinical resistance of these cattle and the amounts of neutralizing antibody present in their sera as measured in tissue culture, using a modification of the titration technique developed by BROOKSBY and WARDLE. No antibodies were found in susceptible controls.

In some animals resistance developed more slowly than in others. Antibody titers might be low 2 or 3 weeks after vaccination, but they might show a rise later and remain as high as those of the animals that had responded faster for a considerable time.

Routinely, antibody titers did not reach a maximum until 4 to 6 weeks after vaccination, although significant titers were found after 1 week.

The techniques presently used for the evaluation of foot-and-mouth disease vaccines, or of the virus suspensions incorporated into them, are not completely satisfactory.

The necessity of employing susceptible cattle is a severe handicap for more extensive work. No other suitable animal is available, however, as the use of guinea pigs has not found general acceptance. The small number of cattle usually employed raises the possibility that individual variation between animals may play an important part in some of the results obtained so far.

Concerning the practical application of the vaccines in the field, several authors have stressed that the use of booster injections may be highly successful to improve responses.

We have been able to confirm this observation completely by means of the neutralization test.

The vaccination of calves still poses some problems, due to the known lack of reactivity to antigens demonstrated by young individuals. According to WILLEMS animals of less than 2 years old should at least receive the same quantity of antigen as adult animals. Application of a booster injection might be expected to help further to fill in this weak spot in our vaccination programs. So far, the role maternal antibody may play by interfering with vaccination has not been investigated.

Total eradication of foot-and-mouth disease should be our purpose. In order to reach this objective international cooperation on a world-wide scale will be essential. It is our opinion that, as long as this goal has not come within reach, it will be necessary to apply large-scale vaccination for the protection of the important numbers of livestock regularly exposed in those regions that cannot be adequately shielded by sanitary measures alone.

The vaccines employed should be of the highest possible potency, but their price should be such that no economical reasons need hamper their widespread application.

Much work has been accomplished towards this aim, but much remains to be done before the problem will be solved completely.

We trust that the new facilities that the United States Government has now made available to its workers in this field will enable these to make a number of major contributions towards this solution.

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COORDINATION OF RESEARCH AND REGULATORY FUNCTIONS

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INTRODUCTION

It is my first pleasant duty to convey greetings from the Canadian Department of Agriculture and the Defence Research Board of Canada to all who are involved in or associated with the establishment of this great institution. Both Departments mentioned have a keen interest in your work although not from quite the same point of view. In addition, I should like to convey the best wishes of my colleagues of the Animal Pathology Division who are engaged in projects somewhat similar to those which are being undertaken here. It is certain that this gathering is a historic occasion and when the pages of time are turned backwards some decades hence the event of which we are active participants will be looked upon as a milestone in the development of scientific endeavours in this great Republic.

THE QUESTION TO BE CONSIDERED

My task is one which has been assigned. I have been asked to speak upon the "Coordination of Research and Regulatory Functions". It should be pointed out that the views expressed are mine and not those of my Government nor of other persons. However, for over 40 years a direct or indirect contact with the field of research and other related work has been maintained and one cannot fail but be impressed with certain features, although impressions are bound to vary with individuals.

Perhaps those who chose my task were aware that before entering upon the study of veterinary medicine training was taken in the fields of philosophy and history. The task is therefore more palatable because in a broad sense it touches these two fields.

NEED FOR RESEARCH

The opening of a great institution, such as this, emphasizes a fact that is easily overlooked, namely, that all the tremendous feats of engineering and the vast construction which are about to form the capital structure are the result of careful planning. Someone had visions. Those have been converted bit by bit into reality. The fact is that all of this planning and construction results from fear--fear which is not to be confused with cowardice--but rather the very real realization of lurking dangers that surround us. This is

in considerable measure the result of the realization that time and space have in large measure been annihilated. When my grandparents came to Canada they occupied 7 weeks on the voyage. The next generation took 14 days to cross the Atlantic. Today, I can step on the evening airplane in Scotland and arrive in Canada the next morning with the rising sun. In a short time the trip will be one of 4 or 6 hours and who knows what the future may bring. That which gave our forefathers protection, namely, the lag between embarkation in the Old Country and disembarkation in the new, has almost disappeared. Infectious agents that may invade animals in the old world have not the long incubation period aboard ship that was formerly the case. Also, infective agents which may be denatured by time may easily arrive in the new world in a viable state. In a word, the fears of our scientific and regulatory personnel are very real indeed and are based on fundamental knowledge and on scientific data. It, therefore, is creditable that this country has seen fit to prepare in advance for that which may come later. Surely this is judicious planning--preparing for defensive war in times of profound peace.

It should not be overlooked that in addition to the accidental introduction of infections that the future may well see the artificial seeding down of infectious agents as a means of destroying an enemy's will to resist. I often think and almost tremble at what could have taken place had our Teutonic enemies been more alive to this. It is said that some of their scientists pointed out the advantage to be obtained from the artificial sowing of disease agents that attack domestic animals. Fortunately blunderers existed in the Teutonic camp as in our own. Consequently, this means of attack was looked upon as a scientific poppy dream, impossible of realization and even if realized of no military value. If at the height of the underseas war as much time and money had been invested in biological agents and their dispersion as in one bomber plane, the free world would have almost certainly gone down to defeat. In a word, quite aside from civilian reasons there is a need for an institution such as you opened yesterday.

SCIENCE AND RESEARCH

Coming now to the subject of research in the field being considered, it would seem that we mean the development of science in relationship to infectious processes. Science is never static. Rarely are advances on a level which precludes improvement. The development of knowledge today only leads to the further development and modification of that knowledge tomorrow. There are two facets of science in relationship to disease; one, the discovery of fundamental principles, the other that which is sometimes called applied science, which is the application of fundamental knowledge in a practical manner. The discovery of a fundamental truth is seldom followed immediately by its application. As a matter of fact, finding a practical means of application is sometimes almost as difficult as discovering the basic truth.

In my opinion, it is desirable to look at science historically, even in a most superficial manner, if we are to understand the different trends in various parts of the world and to realize where these United States stand at present. Doubtless much of the early advance in science took place in Egypt and in those cities that lay in the Euphrates Valley. It is well to note, however, that men did not commence to search for fundamental facts but were attempting to explain that which they already knew. As examples, man learned to smelt metal and make bronze before the days of chemistry and he had learned to reduce a fracture long before the days of anatomy and physiology. Early science had as its objective explaining the background of techniques already known.

A little later when science had become more mature it found a home in the fringe Greek cities of Asia Minor and for a reason which has interest today. On the mainland, there was a great social barrier against those who worked by physical labour. Here the mind was given full play in the abstract field of philosophy; the reason for man and life and his relationship with other individuals. In the fringe cities, however, the aristocracy was composed of traders and those who had accumulated wealth by shipping or other means. The interest of this class related to commercial and other occupations. Consequently, science took root in a practical form and was supported by individuals rather than totally by the State. When the Empire of Alexander broke up and the Ptolemy dynasty was established in Egypt it was fortunate that they were friendly towards embryo science. The discovery of many fundamental facts and the development of techniques based thereon arose at this time. Some of these facts have only been rediscovered within the last 75 years. Here, however, there was a change in that instead of explaining techniques already known an effort was being made to discover fundamental facts; also, the support of science was by the State rather than by individuals. The eggs so to speak were placed in one basket.

We can pass over the depressive period when science almost disappeared from the Western World and note faint signs of its revival in a very unsatisfactory environment. I sometimes think that those who are interested in this period should remember and read again "The Lay of the Last Minstrel". It will be recalled that the wizard Michael Scott -

> "Learned the art which none may name
> In Padua far beyond the sea.
> Men said he changed his mortal frame
> By feat of magic mystery."

Many think that the wizard Michael Scott, who Sir Walter indicated had learned "black art", as it was called, in Padua, is a mythical conception of the poet. Actually, he was a very real individual and

one of the pioneer figures in medicine living about 1200 A.D. Among other things, he appears to have developed a means, which was probably obtained from Persia, of producing anaesthesia by inhalation and under which amputations could be carried out painlessly. Because of this advancement in science he was looked upon as a magician and might well have been kippered for his trouble. The difference is very great indeed in the approach of that day when science was looked upon as something undesirable and related to magic to that of the present day when it is expected to be magic and produce miracles almost over night.

That which must interest us greatly is the rapid growth of science in the English-speaking world, commencing about the Stuart Period. It was then that the Royal Society was founded by such men as Boyle, Willis, Sir Christopher Wren, and Charles II. This in a sense solidified science and cast it in a mold which it was to follow for centuries. One of the interesting features to us today is that research was undertaken by men of means. For instance, Wren was wealthy and Charles II had the revenue of a King. Much of their lives were wrapped up in exploration in one or another of the fields of science, not for material gain but for the very love of the pursuit. Some wag has stated that Charles II courted biology and chemistry in the afternoon and Nell Gwyn at night. Perhaps I may be pardoned for suggesting that his night studies may have had a biological bias.

The important feature of this development is that it was supported by men of wealth and not by the State and that in general the studies were along fundamental lines rather than for immediate application. About the middle of the 19th Century, however, we find that the State commenced to subsidize the research field. In some quarters today where a welfare or semi-welfare state exists the greater part of the financing of research is being undertaken by the State. Here in the United States I think it is true to say that to a very considerable extent you subsidize research out of private funds unless one considers that large corporations are public rather than private. I believe that when science and research become too dependent on the taxpayers' money freedom to a greater or lesser extent disappears. The Alexandrian period which has been mentioned was a period not unlike that which some countries possess today in that scientific research was subsidized by the Ptolemies. When the State lost its interest or for political reasons withheld its support the downward path was rapid indeed. So it might be in many countries where research is almost totally dependent upon Government and where political expediency, or other factors, influence Government support.

From what has been said a false impression might be created. Therefore it is emphasized that the State should also concern itself with research. There are extensive fields which for one reason or another relate in a most intimate manner to the national life of the country. These should not be left to private enterprise but in my opinion are the direct responsibility and concern of the State. Such a field is that which is covered by the work of the institution which you opened yesterday.

It has already been mentioned that research may be divided roughly into two segments - fundamental and applied. The former in turn may be roughly divided into two categories, that type of fundamental research in which no thought of its future application is in mind - the various investigations of natural phenomena are examples. This type of research is generally carried on by universities. It is true that in many instances this work, having no particular practical objective, may yield a great harvest in the applied field. For instance, the investigations in nuclear physics have entirely altered the history of the world. The greater body of fundamental research, however, has in this age a definite objective.

Research cannot flourish in a climate of regulatory function because the day to day problems of the latter are bound to cut across sustained investigation. I do not suggest that the research worker should become the counterpart of a medieval monk. The crux of the situation lies in protecting fundamental research from being pirated by current problems which are more immediately pressing. There is no reason, however, why the two disciplines - research and regulatory function — cannot synchronize since the common bond of interest bridges the gap. The present craze for organization with perpendicular and horizontal lines gives no assistance because it is likely to stifle the efforts of one group or the other.

All this is somewhat difficult to explain to those uninitiated in the research field. The fact is, however, as you who have worked in this field know, there is something about research which is indefinable. The person who undertakes a problem first becomes knowledgeable in the subject, then greatly steeped in it, and finally comes to possess that which is the <u>feel</u> of the problem. The fact is until he becomes so totally immersed in the particular problem under study he is unlikely to make much progress. Once this point is reached, however, he is often in a position to render the greatest of possible scientific service, namely, the elucidation of facts previously unknown. If during this process he is, for one reason or another, diverted temporarily into another field to meet some practical problem requiring immediate consideration he loses and generally forever that most previous of all properties in the field of research, that is, the <u>feel</u> of the problem. I should imagine that this great institution will already have taken what has been discussed into consideration and will have made arrangements so that its scientific investigators, particularly in the fundamental field, will be free from this undesirable feature.

In addition to the fundamental scientist, there is that body of workers who are of equal importance but who are engaged in a somewhat different field of investigation. Their duty is not determining fundamental facts

but determining the solution of problems which arise in application. As an example, there is much to be learned about the effect of various chemicals under varying conditions on the virus of foot-and-mouth disease or, indeed, on any viral agent. Down through the years there has been a tendency to adopt a standard developed for one agent alone and arbitrarily made to apply to other agents. This has been demonstrated repeatedly to be false and the whole field is in need of precise investigation. Here the worker is not greatly concerned with fundamentals but his investigation embraces a more practical problem in which the results may be offered to the regulatory officer for field application.

There is also another segment of the applied field which in many respects is of overwhelming importance. I refer to the problem of laboratory diagnosis. When an unknown disease or a condition which may be of a serious nature and a hazard to the State is being investigated, it is obvious that all methods and tests, which are at present available, should be employed so that a precise and trustworthy diagnosis be made. While it is true that, in general, less extensive methods may serve and only seldom give rise to difficulty, the problems are nevertheless so important that every step should be taken to determine without doubt the nature of an infection that may be an exotic one.

I do not presume to express an opinion concerning what should be done in the United States. Insofar as my own country is concerned, however, I feel that in every instance where the possibility of an exotic disease arises that a thorough investigation should be made and this should include all that science has to offer. The stake is too great to allow any tolerance of error.

Turning now to the regulatory officer, it is unnecessary to point out that he is a most important individual. To be fully competent he must possess a number of special characteristics. He must always keep abreast of the literature and have at least an airplane-view of what is going on in the world of science although he cannot be expected to be competent in this particular field. He must possess a capacity for dealing with the public and above all must have sound horse sense. He, of course, is placed in a position where a great responsibility must be assumed and to meet this should possess a knowledge of where to go for assistance and moreover have a conception of the assistance that may be helpful to him.

There are doubtless a number of ways in which a free flow of material and reports can be shuttlecocked between regulatory officer and scientist. There is no great problem in this except that each must know the limitations of the contributions which can be made and how one may be helpful to the other.

The regulatory officer must use his judgment based on evidence which he views in the field. If, in his opinion, a specimen should be examined then the matter of collection under conditions of safety and a safe

transfer to the laboratory falls within his field of jurisdiction. Once it reaches the laboratory, however, the responsibility for its examination and for all the safety measures which surround an environment of this kind must fall on the shoulders of the scientist. He must be left every freedom to carry out his work as circumstances dictate and without interference direct or indirect. Pressure of various kinds which is brought to bear on him can yield nothing but unhappy results as it may induce an individual who attempts to be obliging to give a premature and perhaps faulty report. As soon as a laboratory decision has been made it must be and almost invariably is delivered promptly to the regulatory officer. From that moment on the results must be read into the whole broad picture. In a word, the regulatory officer who deals with the problem in the field has the assistance of a sound scientific examination in addition to the field investigation which he has made and collectively this gives all the information which can be gathered in the light of science at the present time.

Thus, in my opinion, you have a relationship between research and regulatory function which, although somewhat difficult to define, may be broken down into three component parts. The first is the scientist who is engaged in fundamental work and whose objective is the collection of truths which may be later applied. The second is a group who, in addition to discovering ways and means of practical application of the truths discovered, makes modifications to meet practical requirements. The third group is the regulatory officers who see the condition in the field and have the responsibility of putting into practice the experiences of the past and information developed by the scientists of the world, both past and present.

EPIZOOTIOLOGY OF VESICULAR STOMATITIS[1/]

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Disease is like a baseball game. It exists only in the activity of the participants and the eye of viewers. The confusion of a disease with a virus, or a game with a man, can readily obscure our thinking. The genesis and perpetuation of disease entails not only the survival of the infectious parasite and the susceptible host but the maintenance of environment conducive to their meeting.

The epizootiology of a disease (vesicular stomatitis is the one we shall discuss) is a study of many things—virus, pigs, deer, swine husbandry in Georgia, dairy husbandry in Wisconsin, marketing economics, social customs, game laws, weather, and forage--everything from cabbages to kings. It required the effort of numerous individuals over a period of time. For the past 6 years the research group at Wisconsin, by studies made in the laboratory and in the field, has sought to obtain a better understanding of the epizootiology of vesicular stomatitis. We know more about the disease; we also know that we must learn still more if we are to understand it.

Let us start with the virus of vesicular stomatitis as it encounters a pig, review the disease which results, and then consider the chain of infection.

[1/] Research on the epizootiology of vesicular stomatitis was begun at University of Wisconsin in 1950 under a grant from the Chemical Corps of the U. S. Army. In 1954 support was assumed by the U.S.D.A. A great deal of the work was accomplished only through the cooperation of other agencies--Fort Stewart, Fish and Game Commission of Georgia, Georgia State Board of Health, State Veterinarian of Georgia, State Veterinarian South Carolina, U. S. Public Health Service, and the Wisconsin Conservation Department. The research team at the University of Wisconsin has been composed of graduate students and staff of the Department of Veterinary Science and College of Agriculture.

The virus of vesicular stomatitis, a rodlike particle, 60 mu in diameter and 200 mu long, enters the tongue or snout epithelium of the pig through a small abrasion (4).[2/] It quickly reaches and penetrates prickle cells of the malpighian layer. In a few hours the cytoplasm of the invaded cells is altered and edematous fluid collects between the affected cells stretching the intercellular bridges. Vacuoles appear as fluid accumulates and these coalesce to form vesicles as more cells are involved. In time, large areas of the tongue epithelium may be loosened (3).

The animal at first remains apparently normal. Between the thirty-sixth and forty-eighth hour after infection, virus is released from the cells into the blood stream and into saliva although the vesicles have not yet matured. The body temperature rises to 104° to 105°F by the seventy-second to ninety-sixth hour, when the vesicles have reached their maximum size and virus is no longer present in the blood.

Secondary vesiculation may now be evident. The tongue itself is sore and the animal refuses to eat. If vesicles have appeared on the coronary band of the foot, the animal may limp. The virus has reached a titer of 10^4 or 10^6 infected particles per ml in the vesicle fluid when the vesicle ruptures. Saliva flows profusely and the lips are brought together mechanically in a smacking sound. The body temperature falls precipitously to 101° or 102°F. The epithelium sloughs over a small or large area of the tongue leaving a raw, bleeding surface. Lesions on the snout are more circumscribed. On the foot the vesicles may develop into linear red sores on the coronary band, or they may extend downward loosening the entire hoof and causing it to slough. Repair begins almost immediately and proceeds with surprising rapidity. Virus may be still found in some tissues but is no longer present in saliva. Within a few days even the most seriously affected animals are walking and eating. Most animals are apparently normal in 2 weeks time. High titer neutralizing and complement-fixing antibodies are readily demonstrated and most animals remain refractive to a second infection under natural conditions.

As a disease of swine, cattle, and horses, vesicular stomatitis may cause economic loss to the farmer. Affected animals lose weight and are temporarily unproductive. Cotton (5) reported that in the New Jersey outbreak in 1926 severe lesions appeared on the teats of a majority of the milking cows in two or three dairy herds and permanent injury resulted in a number of animals. A large proportionof the cows had large vesicles or erosions on one or more teats and quite a number on all four of them. Many of the vesicles extended the full length of the teat and sometimes the tip of the teat was sloughed away. Milking under these conditions was a painful operation, both for the cows and for the milkers and, in some cases, it was quite impossible.

[2/]Figures in parentheses refer to list of references at the end of this article.

Heiny (8) of Colorado described an outbreak that occurred in 1943. In one herd of 18 dairy cows, mostly shorthorns, all were affected. There were teat lesions and the udders of these cows were swollen and very sensitive. No milk could be obtained from some of the cows and very little from the others. The owner, in milking by hand, probably aggravated the lesions and spread the infection from one cow to another. As it appeared impossible for any of these cows to recover and to be good milk cows, it was suggested that he dry up these animals and get them in condition for slaughter. The observations of Cotton (5) and Heiny (8) are not exceptional. Brandly (2) reported teat involvement in 2 to 10 percent of some 9,000 milk cattle involved in the 1949 outbreak in Wisconsin. In South America, Strozzi (17) described a Peruvian outbreak occurring in 1953 in which vesicles on the teats and the involvement of the mammary glands were the most pronounced feature of the disease.

Vesicular stomatitis was recognized to be a disease of horses and cattle from the beginning. Although swine were shown to be susceptible to inoculation, the first natural outbreak of the disease in swine was not described until 1941 and then on a Venezuelan farm (1). The appearance of vesicular stomatitis in a hog cholera serum plant in Kansas in 1943 demonstrated the economic consequences of vesicular stomatitis of swine in United States (14). The plant was quarantined, the animals destroyed, and facilities disinfected before operation was resumed. Most of the reports of vesicular stomatitis in swine during the past few years have come from Southeastern United States, particularly the region of Georgia and South Carolina (10). There, the disease is of consequence to the hog farmer insofar as it affects the movement and sale of the animals.

Both Federal and State disease control agencies have been primarily interested in vesicular stomatitis because of its great similarity to two other vesicular diseases, vesicular exanthema and foot-and-mouth disease. The infections caused by all three of these viruses, as they appear in swine, are very similar and cannot be distinguished in the field. Diagnostic field and laboratory tests are required. This may be an animal inoculation test (injection of the tongue of a horse, cow, and pig). Vesicular stomatitis affects all three species, foot-and-mouth disease only the cow and pig, and vesicular exanthema only the pig. In the laboratory, diagnosis may be made by isolation of the virus, by detection of complement-fixing antigens in tissues from lesions or by detection of antibody. Although vesicular diseases are similar in many ways, organized control programs have been applied only to vesicular exanthema and foot-and-mouth disease, they being most serious economically to the farmer. Vesicular exanthema, originally reported from California, has been introduced into other parts of the country yet it appears now to have been eradicated from these areas. Foot-and-mouth disease, an ancient disease of the Old World, has been introduced in United States on several occasions. In each instance a campaign that embraced early detection, prompt slaughter, and terminal disinfection has succeeded in eradicating the disease.

The existence of vesicular stomatitis in any part of the United States, either in an epizootic form in which many animals are affected or in an enzootic form in which few infections are seen, presents a difficult problem to the officials in their attempt to control or to eradicate vesicular exanthema and foot-and-mouth disease. The farmer must be educated to recognize and report any disease of the mouth and foot to a veterinarian and to understand the need for control programs. Cooperation of the local veterinarians must be sought. A more acceptable procedure from the viewpoint of the farmer is needed for handling farm outbreaks during the period that the diagnosis is being made. Quarantine is an effective means of controlling vesicular exanthema and foot-and-mouth disease, but it has not been successful in controlling vesicular stomatitis. Injudicious use of quarantine when the infection is most probably vesicular stomatitis has antagonized both farmers and veterinarians.

The case of vesicular stomatitis described in the introduction may have been one of a few in a community or it may have been one of hundreds or thousands of similar cases. It may have been a mild or severe infection. It may have cost the farmer by reducing either his check for milk or for animals sent to slaughter. It may not have occasioned the farmer any loss. It may have resulted in quarantine involving State and Federal disease control officials or it may have been ignored and never reported.

Epizootiologically, every case has a special meaning because the virus that induced the infection came from a prior case. This infectious chain is the primary subject of this paper.

Every infection goes back case by case in an unbroken series through the years that the disease has occurred. Some links of this chain are relatively simple, some are wondrously complex. Only by tracing the virus through the devious mechanisms of survival during the periods of apparent inactivity (interepizootic period), as well as through the mechanisms of transmission during periods of apparent abundance (the epizootic), can we understand the disease as it is usually recognized. We know much more about the epizootic than we do about the interepizootic period.

Numerous epizootics of vesicular stomatitis have occurred in the United States during the past 40 years. Some of these have involved only a few hundred animals over an area of a few hundred square miles and others have involved many thousands of animals over a region that has included a number of States. One of the first modern epizootics to be described occurred in 1926 in New Jersey and received considerable attention (5). It began in the middle of September and terminated about the middle of November. Some 552 diseased cattle were attacked on 33 farms in an area covering 300 square miles. There were 12 affected horses. Lesions appeared on the feet, the mouth, and on the teats. In 1937 an outbreak was reported which covered northern Wisconsin, Minnesota, and southern Manitoba. The same area was also invaded in 1949, in an epizootic described by Brandly et al (2).

In 1949 vesicular stomatitis was first reported in May in Arizona and then shortly in Texas. Later in the season vesicular stomatitis appeared in 3 widely separated areas in the Southeastern States, the upper Mississippi Valley and in the central Rocky Mountain region. The initial center of infection in the upper Mississippi Valley was located near St. Paul, Minn., in June and from there it spread slowly until late July. Then, during August the disease swept eastward into 10 counties of northern Wisconsin and northwestward across Minnesota and into Manitoba, Canada. When the last of the infection died out in October, most of the horses and many of the cattle in the epizootic area had had the disease, probably 11,000 animals in Wisconsin, 3,000 in Minnesota, and 500 in Manitoba. The disease was often severe. Lesions appeared on the teats of 2 to 10 percent of the milking cattle and in the interdigital spaces of about 50 percent of all cattle. The entire tongue epithelium sloughed in some instances. The infection ran its course in 10 days and produced considerable loss of weight and temporary loss of milk production among infected animals.

In the outbreaks that have been cited and in others that have occurred in United States and in South America, the disease was readily recognized by its affect in one or more of the susceptible species; transmission was presumably direct between affected and susceptible animals. The method of transmission was not definitely established.

We must turn to clues concerning transmission that are evident in the reported epizootics (7). The disease in all outbreaks has spread rapidly among pastured animals and slowly or not at all in stabled animals. It appeared to spread irrespective of trade routes omitting some farms between which there was intercourse and appearing on others which were isolated. The disease characteristically appeared in late summer, spread rapidly during the months of August and September, and disappeared in October. Disappearance of the disease usually coincided with the appearance of hard frosts and snow. Not all affected animals developed clinical signs of the disease. In one Wisconsin herd 75 percent of the animals developed either mouth or foot lesions but all except one animal possessed serological antibodies. Spread of the disease was by no means at random. Certain areas in the country have not reported an outbreak of vesicular stomatitis. Other areas have had repeated outbreaks of this disease. Although the disease has not been reported in southern Wisconsin, it has occurred in northwestern Wisconsin on three occasions--1926, 1937, and 1949--and, in each of these instances, the spread was essentially through the same counties irrespective of the susceptible cattle population in adjacent areas. The disease has recurred many times in certain areas of Colorado and has not been observed in other parts of that State.

Experimental exposure of animals to the virus of vesicular stomatitis has revealed other information about the dissemination of this disease (16). The virus has been swabbed on the intact epithelium of the tongue or gums of cattle without producing infection. The animals did not develop

antibodies and were susceptible to inoculation of the virus at a later period. Ingestion of virus by cattle did not induce infection but virus sprayed into the nostrils of cattle produced an inapparent infection. The animals developed antibodies and were refractory for a short period of time. Injection of virus into the skin, into the muscle, and intravenously also produced immunological response without the development of apparent disease. The introduction of virus into breaks in the epithelium of the mucosal surface resulted in the development of vesicles and typical disease in both adult and young cattle but young calves had a longer course and a pronounced biphasic thermal reaction. Natural infection of calves has been rare; only one animal under a year of age was observed infected in the 1949 Wisconsin outbreak.

Swine differ from cattle in their susceptibility to vesicular stomatitis virus. The virus when fed to adult swine induced, in many instances, an inapparent disease resulting in the development of immunity; in young pigs clinical manifestation almost always followed oral exposure. Abrasion of the mucous membranes appeared to be necessary for the appearance of the typical disease in mature swine. The virus on the intact skin or mucosal surface induced, as a rule, either no infection or inapparent infection. However, if abrasions were present in the mucosal surface or in the skin of the coronary band, the typical natural disease which results in vesiculation was induced.

How are abrasions necessary for entry of the virus produced under natural conditions? Theiler (18), the first to describe vesicular stomatitis of horses, suggested that coarse stalks, spines, and awns of rough herbage might induce breaks in the epithelium for entry of the virus. In the past 50 years there have been other suggestions. Schoening (15) reported that in Wolford County, North Carolina, where an infected herd of swine was studied in 1954, a self-feeder with metal lids was a probable source of injury to the snout; also, crab shells that littered the pen were readily capable of inducing injury to the feet. In his experiments on contact transmission of vesicular stomatitis in swine, Patterson (11) reported that transmission was dependent, in part, upon behavior of the animals. Some, but not all, contact pigs, when introduced into the exposed pen, fought with the donors. Injury to the mucous surfaces generally resulted from fighting; pigs that fought developed the disease or did so more rapidly than those who did not fight. The effect of stones and hardware is obvious. Traum reported in the Foreign Animal Diseases Report, 1954 of the United States Livestock Sanitary Association that large animals were more prone to develop foot lesions.

The action of a biological vector in inducing injury was first suggested by Heiny (8). In 1949, Radeleff (12) pointed out the abundance of the stablefly in an outbreak locality and suggested its consideration as a vector. Strozzi (17) described myriads of the blackfly in the area of a vesicular outbreak in Peru. Experimental transmission of the vesicular stomatitis virus by biting diptera was studied by Ferris (6).

He found that a number of species of mosquitoes and tabanids were capable of picking up the virus and of transmitting it to laboratory hosts for short periods. Since the insects did not remain affected more than 3 days and as there was no intrinsic incubation or host specific it appeared that the transmission was mechanical and not biological. In one trial he infected a susceptible cow by the bite of a horsefly (Tabanus tripeditus).

The probability of an insect vector of vesicular stomatitis appears reasonable. The insect fauna in the area of the 1949 vesicular stomatitis outbreak in Wisconsin was studied by Roberts (13) in order to identify the suspects. He described 32 species of mosquitoes belonging to 7 genera, 15 species of Aedes, 5 species of Culex, 3 of Anopheles, 4 of Culiseta, 3 of Psorophora, and 1 each of Mansonia and Urotaenia. There was an equal wealth of species among the biting flies: 19 species of Chrysops, 13 of Hybometra, 7 of Tabanus, 5 of Simulium, and 1 of Culicoides. All of the identifications were made on collections obtained over a 3-year period from farms on which vesicular stomatitis had occurred during 1949. Not only was there a great variety of biting species of insects which might be considered vectors of vesicular stomatitis, but also differences in the distribution of the species of epizootiological importance. The population in the highland and lowland areas on the farms differed in seasonal abundance in successive years. One species of mosquito, Aedes stricticus, composed 60 percent of the light-trap collections in 1953 whether the insects were collected in low or high land; but, in 1952, it occurred in only 5 percent of the catches in the highland and in 33 percent of the catches in the lowland. In 1952, 64 percent of the season's catch of A. stricticus was obtained during the last week of August near the end of the mosquito season. In 1953, 69 percent of the catch was obtained during the second week of June, early in the season. In the same two years, A. cinereus had two population peaks, one early in June, one late in August. The light-trap collections, of course, are not a true index of relative abundance of species which bite pigs, cows, or man. In 1953, when A. stricticus made up 60 percent of the light-trap collections, it constituted 82 percent of the bite records. It is evident, nevertheless, that mosquitoes are found throughout the vesicular disease season, some species being more prevalent during the early part and some during the later part. On the basis of ecological and seasonal distribution, it would be possible for mosquitoes to act as vectors of vesicular stomatitis. The tabanids, while found in pastures and are vicious biters, have their primary period of abundance from about the 15th of June to the 15th of July in northwest Wisconsin. On the basis of their prevalence, it would appear that mosquitoes would be more probable vectors of vesicular stomatitis in Wisconsin than the tabanids.

Helminths are animals that can produce abrasions of the mucosal surfaces. Karstad (9, unpublished) fed swine preparations of vesicular stomatitis virus. One group of swine received only the infected feed and the other received embryonated ascarids along with the virus infected feed.

In both groups of swine vesicular stomatitis antibodies were demonstrable after a period of 2 weeks but only in the group that received the embryonated ascarids did the temperatures rise and vesicles develop. It is quite possible that some of the helminths may act in producing abrasions and make possible clinical infection of swine.

We may postulate that an epizootic of vesicular stomatitis will develop whenever a carrier animal is brought into an area in which the vectors, presumably some of the diptera, are present in sufficient numbers. The vector obtains the virus from the infected animals and injects it into the mucosa of susceptible animals. The abundance of the disease during an epizootic coincides with the abundance of certain vectors and termination coincides with the disappearance of these vectors. For the vector to be effective it should commonly feed on the contaminated surfaces, it should be capable of interrupted feeding, and it should be a relatively strong flier. Mosquitoes and tabanids, to a certain extent, fit these requirements. It has been observed that in the epizootic the affected cattle generally have been kept in wooded pastures. This would suggest tabanids and certain mosquitoes. Until vesicular stomatitis virus is isolated from insects during an epizootic, the question of the vector or vectors cannot be determined.

In our sketch of vesicular stomatitis we have delineated the part picturing the disease in an individual animal and the part depicting transmission during an epizootic, but the picture remains incomplete until we can fill in that part concerning the interepizootic survival. Our first step is to distinguish the enzootic from the epizootic areas. The enzootic areas are those in which the disease recurs annually and the nonenzootic areas are those in which the disease occurs at infrequent intervals, sometimes 10 to 20 years apart. There are some definite ecological differences between the enzootic and the nonenzootic areas. All of the known enzootic areas lie in the South, in the countries surrounding the Caribbean, alongthe southern Coastal Plain of the United States, in the region of the Carolinas and Georgia, and possibly in an area along the Gulf of Mexico. The nonenzootic areas in which epizootics occur are found in the Appalachians, the upper Mississippi Valley, and in the Rocky Mountains. If one were to make a serological survey of animals in these two areas, one would find certain definite differences. One-hundred percent of the horses were found to carry antibodies in the enzootic area of Georgia, approximately 50 percent of the cattle, and about 75 percent of the swine. If one were to go into the nonenzootic area, for example, northwestern Wisconsin, during an interepizootic period, antibodies would not be found in swine and antibodies would be found in cattle or horses of the age group that passed through the last epizootic. As high as 50 percent of those surviving 7 years after an epizootic have been found to have antibodies. All replacement animals born since the outbreak would be negative. The antibody persisting in recovered animals in the nonenzootic area, as determined by the serum neutralization or by the complement-fixation

test, is not present to the same titer at all times in the sera of an individual animal. Over a 5-to 7-year period the titer periodically fluctuates from high to low but does not decrease appreciably. This might lead one to suspect that such animals are potential carriers of vesicular stomatitis. Nevertheless, all calves born to these previously exposed cows, although receiving colostral antibodies, or cattle that have been introduced into herds in which these reactors occur, have not developed neutralizing or complement-fixing antibodies to vesicular stomatitis. Clinical evidence of the disease has not been observed. On the basis of this evidence, it does not appear probable that cattle, swine, or horses are effective reservoirs of vesicular stomatitis. The next step is to consider other animals that might be long-term carriers of vesicular stomatitis virus. Sheep and goats appear to be refractory to natural and artificial infection. The domestic chicken is susceptible to the virus inoculated into the tongue mucosa but the infectious period is short and antibody production quickly follows. Wild animals might be considered carriers of the disease. In studies made in the enzootic area of Georgia approximately 50 percent of the deer have been found to carry antibodies to vesicular stomatitis. In epizootic areas in Wisconsin antibodies have not been found in deer. Vesicular stomatitis antibodies were demonstrated in raccoon in Georgia. They are not found in raccoon in Wisconsin. Another animal observed to have antibodies to vesicular stomatitis in Georgia was the bobcat. Although all of these animals have antibodies to vesicular stomatitis and, therefore, must have been infected with the virus, the important thing as far as the enzootic is concerned is whether or not they are capable of carrying the virus for any period of time and transmitting it to other animals. Experimental infections of deer and of raccoon have shown that they develop a short-term infection, somewhat similar to that of swine. The deer is quite sensitive to the virus. Quantities too small to be detected in cattle have induced vesicles in deer. The lesions heal rapidly and antibodies are produced. The raccoon develops an inapparent disease, as a rule, followed by an antibody response. On the basis of experimental infection, it does not appear that either the deer or the raccoon play an important role as a reservoir of vesicular stomatitis although they may add fuel to an epizootic of the disease.

Since deer, raccoon, bobcat, horses, cattle, swine, and man are all exposed to vesicular stomatitis in Georgia, it appears that there must be a common source of infection. There must be some relationship between these animals and the hypothetical carrier, or between these animals and a vector which has access to the hypothetical carrier of vesicular stomatitis. Studies of the vector are being continued. In the meantime, the susceptibility of various species of animals, vertebrate and invertebrate, are being tested in search for a reservoir. Since swine are frequently the first animals in an area to be infected with vesicular stomatitis and since swine, bobcats, and raccoons prey upon lower animals, the possibility of a virus carrier among one of the food species is being investigated.

The earthworm, for example, is eaten in large quantities by both the pig and the raccoon. As many as 200 earthworms have been taken from the stomach of a feral pig in southern Georgia. Vesicular stomatitis virus was not isolated from one of the most common species of earthworms to be found on an infected premises.

Frogs and crayfish are common throughout the enzootic area. A finding of possible significance is the induction of a "carrierlike" state in a frog which persisted for 6 weeks. Frogs fed the virus and kept in a state of hibernation still contained infective vesicular stomatitis virus 5 to 6 weeks later.

A search for the reservoir in which the virus persists during the inter-epizootic periods and for the vector which spreads the virus during the epizootic continues. Certain clues have been provided by the extension of the known host range of vesicular stomatitis among warm-blooded animals. Knowledge of their habits, of their parasites, of their other relationships are of assistance in sifting through the great variety of species of small animals for the one or ones which are the important reservoirs or vectors of vesicular stomatitis virus.

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SOUND RESEARCH WILL HASTEN THE END OF COMPROMISE WITH DISEASE

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Research in the field of animal diseases and parasites is of basic importance to the national well-being. Research supplies, the knowledge for detecting and combating animal diseases and parasites to provide healthy livestock and poultry. Healthy flocks and herds are imperative if the farmer is to profit from the sale of animals, animal products, and animal byproducts. He must make a profit if there is to be a progressive, prosperous agriculture on which to build a sound national economy, a high standard of living, and a healthy population.

A nation's flocks and herds must be healthy if its agriculture is to supply wholesome, disease-free food. They must be healthy if the quality of animal products is to be provided in adequate quantity to support a high standard of living. They must be healthy if the efficiency of food-conversion is to be increased to provide a more profitable utilization of tillable and pasture lands. This is extremely important to the over-all agricultural economy when one realizes that millions of acres of grazing land, together with approximately 58 percent of the acreage under cultivation in the United States, are used to produce livestock and poultry. Excess lands that are made available could be placed in a soil conservation program for controlling surplus agricultural products at the present time. Nevertheless, the potential of resources for increased production would be available for future needs and national emergencies.

Continued research can provide the answer to these challenges. It can provide better quality of animal products in adequate quantity, at reasonable cost and fair profit. And the job can be done with fewer animals and less feed.

The significance of research on animal diseases and parasites cannot be intelligently appraised without recognizing the close relationship between animal and public health. This is emphasized by the fact that more than 80 communicable diseases and a larger number of parasites and parasitic diseases are transmissible from animals to man. These cannot be prevented in people unless they are eliminated from animals which are the primary hosts. Any contribution to animal health is inevitably a contribution to human welfare.

These then are the broad objectives toward which animal disease and parasite research is directed. But the present status of many animal

diseases throughout the world points out the urgent need for further research to provide the necessary information to obtain the goal of control and eventual eradication. In the United States at this time we are controlling or partly controlling some highly contagious and infectious diseases by the use of modified living vaccines. Of these diseases outstanding examples are brucellosis, hog cholera, Newcastle disease, rabies, and anthrax.

Before an intelligent approach can be made to control and eventually eradicate infectious diseases, however, much additional work has to be done in the fields of diagnosis, mechanism of infection, immunology, tolerance, and treatment. To be more specific, special emphasis must be put on determining methods of transmission, identification of inapparent carriers of infection, ecology, epizootiology, host adaptation, and the development of safer, more practical immunizing agents. It would be highly desirable to develop and employ innocuous immunizing agents in order that the propagation of the specific disease-causing agent could be entirely eliminated.

Research in parasitology has been directed successfully toward increasing knowledge of the occurrence, distribution, life cycles, and methods of coping with parasites and parasitic diseases of animals. Among the more important results that have stemmed from facts developed in the course of these investigations are (1) the importance of sanitation in raising livestock and poultry; (2) development of some effective medicinal treatments for the elimination of internal parasites from cattle, sheep, swine, poultry, and other animals; and (3) the development of methods of destroying the vitality of trichinae and other injurious parasites of meat by refrigeration, heat sterilization, and other processing procedures.

The possibility of breeding disease-resistant livestock and poultry is a field of research that might be profitably explored. There is considerable indication in the literature that certain breeds of animals are more resistant than others to certain diseases. Also, claims have been made that certain cross-bred cattle are more resistant to certain diseases and parasites than are the parent breeds from which they originated. Claims have been made that one strain of sheep showed greater resistance to stomach worms than others, but the evidence presented to substantiate these claims has not been impressive. The problem of development of disease-resistant strains of livestock and poultry is a very difficult and complex one. However, the testing of different breeds and strains within breeds for resistance to infections is a challenging and promising area of research.

There is a need, also, for a more complete analysis of the objectives of related research fields to stimulate research which will provide information that will assist in controlling livestock diseases and parasites. For example, engineering research is directing research

studies on the design of buildings, facilities, and equipment to minimize the propagation and spread of diseases and parasites. Studies on this problem concern drainage, ventilation, and isolation, and selection of materials and designs which lend themselves to various types of disinfection or sterilization.

The need for sanitary housing has led to the development of a system of raising dairy calves in the Southern United States in portable pens because of coccidiosis, various worm parasites, and other fecal-borne diseases. Another example is the development of farrowing houses for swine of such construction that makes it possible to clean and disinfect them with hot water and lye to destroy ascarid eggs and disease agents that are dangerous to young pigs.

Research in technological and managerial practices can help to combat those parasites and diseases that cannot be alleviated by known methods of medication. Before such practices could be evolved, it would be necessary to have precise knowledge of: (1) The ecological relationships of parasites, their life cycles, and the manner in which they damage the health of the host; (2) the length of time the infective stages of parasites or the infective agent can persist on and in soil and in carriers or intermediate hosts; (3) the effect of such practices as crop rotation, pasture rotation, plowing, and fertilization on the persistence of infectious agents or parasites on pastures; (4) the behaviour of the disease agent or parasite on pastures, in barns, and elsewhere; (5) means of circumventing infections by chemical treatment of pastures, sanitation of barns, and control of vectors; (6) methods of alleviating the effects of the infections by medication, nutrition, immunization, and other means, all of which constitute a part of managerial problems. The best procedures have not been developed or adapted to farm practices.

These, then, are merely examples of how an interrelated approach to the problems of disease prevention, control, and eradication helps to bring more effective results. All phases of livestock production have an influence on either decreasing or increasing the effectiveness of disease control. By coordinating research planning and findings, it is possible to move consistently toward increasing that effectiveness.

In considering research on diseases and parasites themselves, an analysis of where we stand today might logically be broken into 3 areas of discussion: (1) Areas of relatively adequate research coverage, (2) areas of inadequate research coverage, and (3) areas of substantially no coverage. To consider each in turn, we find some fields in which the work being conducted by public and private research is giving relatively adequate coverage to the needs as they can be seen now. The coverage cannot be considered complete because investigations have generally dealt with only the more urgent problems within the specific research areas. But, at least, themajor problems are under study.

In this field, some of the major aspects of diagnosis are adequately covered. The first step in diagnosis--the isolation, characterization, and classification of causative agents of infectious disease--is given considerable attention. This is true for bacterial, mycotic, viral, and rickettsial infections. Also, the study of the morphology, taxonomy, and identification of parasites as causative agents is relatively adequate. Attention is being given to diagnosing conditions caused by metabolic disturbances, poisoning by heavy metals, insecticides, herbicides, and poisonous plants.

Serological tests are being developed and improved to identify a specific response of the animal's body to specific agents. Examples of this work are the successful tests for brucellosis, Salmonellosis, Newcastle disease, foot-and-mouth disease, toxoplasmosis, Q-fever, and anaplasmosis.

As another aid in diagnosis, studies are being made of the prevalence and distribution of livestock and poultry diseases the world over, and references are being maintained on the world's literature in veterinary zoology.

Allergic responses are studied to identify animals exposed to infectious agents. Examples of such effective methods of identification are those for tuberculosis, paratuberculosis, and toxoplasmosis.

Chemical analyses are made of body tissues and fluids, as well as of feeds, to identify poisonous substances responsible for pathological conditions in animals. These analyses have lead to the identification of causative agents for hyperkeratosis, fluorosis, heavy metal chemical poisoning, plant poisoning, herbicide poisoning, ketosis, and other conditions.

Methods have been developed for histopathological evaluation of affected tissues revealing changes that are indicative or confirmatory of specific infectious, noninfectious, and parasitic diseases.

Data have been gathered to demonstrate patterns of clinical symptoms for many infectious, noninfectious, and parasitic diseases--some showing specific patterns, others suggestive. Examples of such diseases are milk fever in cattle, bloat, laminitis, rabies, pseudorabies, trichostrongylosis of sheep, lungworm disease, and scabies.

Definite progress is also being made through research in developing knowledge of immunology, tolerance, and treatment. Immunizing agents serve a purpose in lowering incidence of some infections when they are endemic and widespread. But no immunizing agent, used without proper management and sanitation methods, has ever eradicated an animal disease. Examples of relatively successful immunizing agents, used within the limits of their purpose, are those for brucellosis, equine encephalomyelitis, rabies, blackleg, anthrax, hog cholera, pox infections, Newcastle disease, and laryngotracheitis. However, their greatest value in the control of disease is an adjunct to sound management and sanitary practices.

Studies have demonstrated the tolerance of animals to insecticides, herbicides, and some of the other chemicals commonly used in modern agriculture.

Research has been conducted to determine the prophylactic and therapeutic values in combating animal disease by antibiotics, endocrine derivatives, and other new drugs. Part of these studies have been directed toward the development and standardization of treatments for the removal and control of serious protozoan, helminthic, and arthropod parasites. Some antibiotics are highly specific in certain types of infections, however, in many infections their greatest value is an alteration of the course and intensity of infection.

Methods of processing meat and meat products for food have been developed to destroy parasites and to make such products safe for human consumption. Examples are the methods of destroying trichina and tapeworm cysts in animal carcasses.

Another area of relatively adequate coverage is research on mechanism of infection. Continuing investigations are being made of the causative agent, incubation period, course of infection, clinical manifestations, methods of transmission, and reservoirs of infectious disease. Also, in parasitic infection, research is being carried out on the mechanism of infection, mode of transmission, and other aspects of the life history of parasites.

Studies are being conducted on bionomics of the free-living stages of parasites of livestock and poultry and of their intermediate hosts.

In the second phase of the analysis of today's research on animal disease and parasites, we come to areas of inadequate coverage.

More work is needed on chronological histopathologic studies of disease processes under controlled experimental conditions. There is little doubt that information developed from such an approach will provide a much better understanding of diseases.

Not enough is known about growth requirements of many parasites and infectious agents. For example, the recent adaptation of tissue culture for propagation of microorganisms and a study of their effect on the living cell have the potential of a better understanding of disease processes and immunity.

Study is only beginning on the toxicology of new antibiotics, drugs, insecticides, and herbicides. Additional drugs and materials are constantly being developed, even faster than research can determine the full effect on animals of the ones now in use. Closely related to this need is the requirement for more thorough assays of residual deposition of chemicals in body tissues and fluids and their effect on products from livestock and poultry.

There is a need for continuing and wider investigation of the pathogenesis of specific infections.

Pharmacology, toxicology, detoxification, and the mechanism of the action of antiparasitic drugs should be studied more thoroughly to provide adequate information in this field. Furthermore, we need an expansion of basic researches on theory, principles, and methods of chemotherapy and on designing programs for disease and parasite control.

We should know more about the economic significance of subclinical parasitism of cattle, sheep, and swine in relationship to control measures to be recommended.

Merely a beginning has been made in the research on the relation of nutrition and general well-being to susceptibility of livestock and poultry to disease and parasites and the relation of these factors to the extent of damage caused by disease and parasites.

Research should be expanded on the value of environmental control of diseases and parasites. These studies could be broken down into four phases: (1) The effectiveness of chemical destruction of agents on pastures and premises; (2) the development of additional knowledge about infectivity, virulence, and reproductivity of agents of infection; (3) additional information on vector control of livestock diseases, especially arthropod-borne diseases, with studies of parasite relationship to epizootiology of special diseases; and (4) further investigations of controlling diseases and parasites and parasitic diseases by management and engineering practices.

Today's research is also inadequate on the study of the chemistry of infectious agents and the relationship of this factor to their ability to invade and infect body cells.

We should know more about the chemical changes of body tissues and fluids in disease processes as well as in immunological response.

To provide more effective and specific diagnostic tools, there should be further study in fractionation of infectious agents and antibodies.

In developing control programs it is important to know more about the effect of wild animals and birds as reservoirs of disease agents and parasites affecting livestock and poultry. In developing control programs for disease, standards for diagnostic procedures should be determined for all serious infections as well as tools to cover new disease conditions which arise.

Efforts should be extended to preserve microorganisms and body tissues and fluids to make them readily available for long-range studies.

In the third phase of the analysis we find areas in which there is substantially no coverage in the research conducted today on animal diseases and parasites. Knowledge in any of these fields, now virtually untouched, could provide valuable aid in reaching the final objectives of our research.

For instance, here in the United States very little research of any consequence has been conducted on exotic diseases of livestock and poultry. The effects of such agents on our livestock and poultry are of great importance. Until the opening of this laboratory on Plum Island, no research on infectious foreign diseases had been conducted. We can hope now that at least a part of this deficiency will be met.

There is little information being developed on the physiology of the host-agent or parasite relationship, including the physiology of the agents and the alterations which these agents produce in the host.

Knowledge should be developed on the role of metazoan parasites as vectors of viral, bacterial, protozoal, and other diseases.

There is a great need for the expanded application of physical science to the development of equipment especially adapted for use in research on animal diseases. Examples of equipment which should be more widely used are electron-microscopy, electrophoresis, lyophilization, special germicidal equipment, ultracentrifugation, filtration, adsorption, and sonic vibration.

We need to further develop a definite characterization of immunity and methods for measuring this quality.

We need to know the relationship of genetic factors as well as that of the endocrine function to susceptibility and/or resistance to disease.

Work should be carried on to study the effect of antibiotic additive diets on the response of animals to infection and immunization.

Furthermore, a relatively large number of livestock and poultry diseases are causing extensive losses in the United States today. Sufficient knowledge of such diseases are not available to control their spread. There should be extensive research on such diseases as scrapie, mucosal disease, most mycotic infections, rhinotracheitis, infectious keratitis, vaginitis, ketosis, gut edema, bluecomb, white muscle disease, and many parasitoses.

In these areas of research in which there is inadequate or substantially no coverage, there are serious handicaps to expanding the work through lack of personnel, equipment, and facilities. However, progress in such expansion is so important to food production, agriculture, and the world standard of living that a way around these handicaps must

be found. Ways must be found to increase scientifically trained personnel engaged in public and private research, possibly through increased educational opportunities in the fields of science. Ways must be found to increase equipment and facilities and to expand the use of the facilities now available to this research. These problems must be solved if animal disease and parasite research is to fulfill its responsibility in the world of the future.

Sound research will hasten the end of compromise with disease!

Greetings

from

G. Ramon

International Office of Epizootics
Paris, France
(read by G. A. Moosbrugger)

I am highly honoured to be here today and to represent Professor Ramon, whose many commitments have prevented him from coming himself. He has asked me to tender you his apologies and to convey to you on his behalf the congratulations of the International Office of Epizootics and every good wish for your success.

You will already have noticed that I have my own exclusive brand of English pronunciation which cannot be classified with any of the seventy-seven forms recognized in Great Britain or with any of the seventy-seven times seven forms known in the United States. I hasten to assure you, therefore, that I shall be brief so as not to overtax your powers of comprehension and that I shall stop before the majority of you have succumbed to exhaustion.

Every spring, as regularly as the birds lay their eggs, the International Office of Epizootics issues a certain number of recommendations. With the same satisfaction as the bird must experience on seeing its eggs hatch, I should like to express my pleasure at seeing one of these recommendations taking concrete form. The creation of a research laboratory for foot-and-mouth disease must be encouraged and the day it commences work should be celebrated as an important event in the history of scientific progress. There are three reasons for this.

In the first place, our own angle of vision is very restricted when we attempt to define the aspects of the problems with which we are engaged. We must, therefore, try to see our problems from as many angles as possible in order to understand the whole. We are rather like an architect who wants to restore a Gothic cathedral with the aid of photographs taken at a distance of 3 feet from each subject. The details are clear enough, but the general plan eludes him. We have no doubt that Plum Island will supply us with important elements which have hitherto gone unobserved.

Secondly, the control of foot-and-mouth disease on an international scale presupposes an active and all-out campaign in each country. The International Office of Epizootics congratulates you on having completed your over-all defensive preparations by creating an organization such as this, which is indispensable if prophylaxis against the disease is to be really effective. Although the strict barriers you have set up have protected you so far, the experience of your neighbours has demonstrated the inadequacy of such measures. The institute you are inaugurating today crowns a work of which your country may be legitimately proud.

Thirdly and finally, we who are combating the disease are happy to know that you stand by our side. Until now we have been like swimmers trying to push to the shore a heavy raft without handles for us to grip. You shouted advice and encouragement from the bank, but now you have leapt into the water with us and there is between us that solidarity felt by people who are all "in the swim" together.

Any speech worthy of the name consists of three points; thus, I may now pass to the conclusion. With modern traffic as heavy as it is, the fight against epizootics must be international if it is to be waged at all. Your national work is an important contribution to the general campaign. The International Office of Epizootics is grateful to you and it is confident that Plum Island will prove to be, for the good of all, one of the finest achievements of a democracy which already has so many to its credit.

THE DISSEMINATION OF FOOT-AND-MOUTH DISEASE BY AGRICULTURAL PRODUCE

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Once the real nature of infectious diseases has been recognized, it is possible to study the problem of their dissemination and elicit its principle. Research undertaken in this field, however, has quickly shown that a formidable complexity often lies masked beneath the apparent simplicity of a problem. If in some cases, such as anthrax and its "champs maudits", a solution has been immediate, in others the processes of virus dissemination are still the subject of hypothesis or even remain virtually unknown or incomprehensible.

Foot-and-mouth disease is one of the diseases in which the study of contagion has presented the greatest difficulty, and it may even be claimed that no final solution has yet been found to the problem. It is the purpose of this paper both to summarize the established facts and to indicate the points still to be elucidated. Certainly direct dissemination is, in the vast majority of the cases, as regular as our systematic minds could wish, although certain exceptional instances met with in the field may strain one's credulity. Indirect transmission is often so highly irregular that even today it gives grounds for and encourages belief in medieval superstitions. This holds good not only for contamination itself, which is variously ascribed to the air--atmospheric or, for that matter, stratospheric--or to unknown radiations, but also for the prevention of the disease, which is procured by the most unexpected means substantiated by an abundance of proofs.

It is beyond dispute that the essential characteristic of foot-and-mouth disease is its startling contagiousness. If it is remembered that Möhlmann had little difficulty in reaching an infectious index of 10^{-15}, it must be admitted that this cannot be far from a monomolecular reaction, that is, a single molecule of virus is enough to produce the disease. On the other hand, it is in our power to prevent any contagion by comparatively simple methods. We also know that in practice all the recognized possibilities of transmission by indubitable contacts are not regularly followed by an outbreak.

It is to this discrepancy between the laboratory and practical experience that we must first give our attention, for in our view it has been neglected too long and still awaits proper study. First of all it must be asked whether the contagiousness of foot-and-mouth disease is purely quantitative, entirely qualitative or, which is the most likely, a variable combination of the two preceding modes. In other

words, to return to Möhlmann's tests, they may be understood either in terms of an increased multiplication of the virus or of a modification in its structure. The test in itself does not allow us to decide one way or the other, and yet it involves the simplest case where the virus passes almost directly from a diseased tissue to a receptive healthy tissue. In the case most frequently met with in practice where the same virus is first of all expelled from the organism, where it is exposed for a time to external influences such as light, desiccation, alkalinity or acidity whose full action is unknown, it is far more difficult still to decide between quantitative and qualitative. A typical example is provided by the strain known as A5 (A7 Ubertini) which ravaged Europe in 1951-52. In the field this strain displayed an almost unprecedented power of dispersion, being disseminated over incredible distances and to the remotest of spots. In the laboratory its virulence proves to be low, as may be shown both by the dilution method and by observation of the hundreds of virus donors required in the manufacture of the vaccine. In fact a large proportion of the latter, despite the high concentration of the suspensions of infective agents, regularly displayed only isolated aphthae, the majority of the points of infection not reacting at all. Thus a virus, apparently highly contagious as revealed by a study of its epizootiological behaviour, showed itself to be only weakly contagious quantitatively. It must be admitted that it was of its own nature very insensitive to the forces which to a great extent destroy the virus outside the organism.

The observations made in France in 1955 and in Switzerland in 1956 with a virus of type O are no less interesting. This virus, without having a marked invasive character, was disseminated from cowshed to cowshed, proceeding in jumps, affecting only a few farms in each village. What is even more curious, often only a few animals in the cattlesheds were contaminated. All the same, it went on for months in France and it took measures as drastic as those of 1951 to stamp out the epizootic in Switzerland. These two examples will reveal in all clarity the fundamental difference between quantitative virulence that can be established in the laboratory and the qualitative infectious potential observed in practice. It is the latter that is important in the prophylaxis of epizootics. We shall see that in certain cases the insidiousness of the virus far exceeds what one would imagine simply from the tests carried out in the laboratory.

Combating an epizootic involves nothing more or less than artificially interrupting the normal cycle of infection, virus source, and transmission to the susceptible organism which in turn produces the virus. In theory the break can be made at any point in the cycle. In practice it is at the same time a question of what can be achieved under favourable conditions. Cattle plague, for example, has been eliminated from Europe by the wholesale slaughter of the beasts infected on the appearance of the first symptoms; Australia has been able to

protect herself against several epizootics by the stringent control of imported produce; smallpox has disappeared from continental Europe by the general use of vaccination, which removes the susceptibility of organisms normally liable to infection.

Conditions are not always favourable. In spite of slaughter and vaccination, swine fever (hog cholera) has not disappeared entirely. Rabies, which in Europe was no more than sporadic--in 30 years only one single outbreak had been observed in Switzerland and that was in a frontier region--is at present spreading in Germany, especially amongst wild animals.

Foot-and-mouth disease is also one of those diseases which, in spite of the best methods devised, constantly makes a reappearance and which may easily turn into a disaster. Experience shows that in the case of this epizootic any attempt to interrupt the cycle at one point only is doomed to failure. By its very nature the elimination of the virus source is always achieved too late when the infective agents already have been disseminated; the measures taken by the sanitary authorities must necessarily leave loopholes unless economic life is to be completely disrupted; lastly vaccination does not yield 100 percent results, and the few subjects which for one reason or another do not respond are amply sufficient to disseminate the infective agent. Thus it is essential to attack all the weak points at the same time in order to compensate for the failures, foreseen and foreseeable, of the measures aimed at one or the other individually.

The insidious power of foot-and-mouth disease virus to spread contagion gives rise to problems that are extremely difficult to master as soon as it is desired and the need is imperative to prevent its transmission. We have said that the measures imposed by the sanitary authorities for the very purpose of preventing all transmission of the virus leave loopholes. In fact, the requirements of economic life preclude a standstill of all traffic, for traffic is essential to its existence. A choice will have to be made, that is to say, a compromise between two alternatives--bringing to a standstill the movement of all persons and objects capable of transmitting the infectious agent or allowing trade to take its normal course without impediment. The choice must be made with a full knowledge of the facts and that is why a study of the dissemination of the foot-and-mouth disease virus was one of the first to be undertaken. The fact that it has not yet been concluded after half a century of effort points to the complexity of the factors involved.

Let us say, once and for all, that the most important disseminating agent, particularly inside a country, is man himself, for he is unquestionably the most mobile and the least restricted of all the carriers of the virus. Whereas insects have a well-defined radius of flight and vertebrates a clearly delimited territory, man moves about without let or hindrance. He has possibilities of spreading the disease directly and indirectly over distances, graphically illustrated by the appearance of foot-and-mouth disease in Canada.

Immediately next to him in order of importance and by their very nature comes agricultural produce. That is why no time was lost in studying the survival of the virus outside the organism. In order to make our investigation as comprehensive as possible, we will divide it into two large groups.

1. Animal Agricultural Produce

a) Living animals. Although there is no need for us to dwell on the transmission of the virus by animals that are clinically diseased, two cases demand our attention. The first concerns animals that are in the period of incubation, that is, before the appearance of any visible symptom. It is necessary to discuss this because it may have a part to play particularly in the slaughterhouses. In point of fact, and contrary to what has been published thus far and has almost come to be regarded as a dogma, the virus does not pass into the blood after the formation of the first aphthae; it can be shown to be present there much earlier. We have found it in the blood of an animal nine hours after intralingual inoculation with an O strain of virus, that is, several hours before the formation of the first aphthae.

The second case concerns animals that recovered from foot-and-mouth disease and no longer exhibited any symptoms. It is the problem of the permanent excretors or germ carriers. In the laboratory only Waldmann and his collaborators have succeeded in demonstrating the presence of foot-and-mouth disease virus in the urine of animals infected several months before. We are aware that doubt has been cast on these results by several workers and we do not hesitate to admit that the accounts of the experiments published are not in themselves entirely convincing. If observations made in practice are so often vitiated by sources of errors that they must be interpreted with the greatest caution until experimentally verified, care must be taken not to fall into the opposite error of denying them a priori any demonstrative value. If in Switzerland we have employed the slaughter policy since the beginning of this century, it is because time and again contact between animals cured for several months and fresh animals gave rise to new foci of infection. Moreover, from the epizootiological point of view, it is of no great importance whether the infectious animal is actively infectious, that is, it produces the virus permanently or intermittently, or whether it is only a passive agent that transports the virus which is found in its shed and has preserved its contagious activity. If in our personal opinion the first possibility is the more likely, we recognize that in many cases the second cannot be finally excluded. The important point is that we have been able to observe transmissions of this kind up to 27 months after recovery. That is the only thing that matters in regard to the eradication of the disease or the protection organized against its introduction. On the other hand, and in spite of the millions of vaccinations carried out, no authentic case of transmission of foot-and-mouth disease by a vaccinated and noninfected animal has been observed to date.

b) Meats and meat produce. The outbreak of foot-and-mouth disease in a country immune hitherto as a result of the importation of frozen meat is a fact so well established that there seems to be no useful purpose in considering it further. Nevertheless it does frequently reappear for discussion in the hope, the vain hope, that new arguments, which are no more convincing than their predecessors, will succeed in revealing a fallacy. But we cannot combat a danger by denying its existence. Moreover a sharp distinction must be drawn between meat that has been chilled slowly after slaughter and by this process has acquired an acid pH value that kills the virus without fail, and meat which, as the result of deep and rapid freezing immediately after cutting, is in an optimum condition for preserving the virus. All the same, if in the first case the muscles are sterilized, it is not always certain that this applies to the marrow of the bones, the lymphatic glands embedded in the fat, or the surface of the aponeuroses which the acidification does not reach. Nevertheless, in practice, the virus takes a weakly aggressive form and can only manifest itself if supplied with favourable or even optimum conditions. This is why these foci are limited almost exclusively to the contamination of pigs fed on undercooked scraps. Since the diagnosis of foot-and-mouth disease is not always easy in the case of the pig, a further spread is always to be feared and experience shows that it is not rare. It should be pointed out here again that economic requirements are paramount and that the danger of infection is accepted whether we like it or not if the country's supplies of beefsteaks demand it.

c) Hides and skins. Dissemination by skins plays a very minor role, for tanning invariably kills the virus. All the same, some outbreaks have been observed where scraps of raw or green skins have been fed to pigs. It is moreover simple in this case to prevent all dissemination, for salt alkalinized with 5% common soda is completely effective as a disinfectant in a week. This is rather curious, for pure sodium chloride has a marked preservative effect on the virus although it is generally slightly acid.

d) Manure. From a national as well as an international point of view, the dissemination of the virus is rare in its direct form, for this is not a product with many commercial outlets. On the other hand, before the disease is recognized or declared, animal droppings carried on to the fields may infect considerable portions of the roads, especially in winter, and from there be carried very great distances by the cars of tourists. Time and again in Switzerland we have observed these road infections which have introduced foot-and-mouth disease from abroad into regions which were completely free.

Immediate measures are taken to isolate the infected area and if these measures are strictly adhered to, the danger disappears, for

the disinfection of manure is a problem which was solved long ago by the use of auto-sterilization. Nevertheless it should be borne in mind that this method is effective only in depth and that the surface may remain infectious.

To summarize, animal produce, with the exception of germ carriers which are, if not always impossible, at least very difficult to track down, is the source of relatively few dangers because it is always possible to take defensive measures against them and neutralize them to a very large extent.

2. Vegetable Agricultural Produce

This produce, whose importance has come into prominence particularly during recent years, plays a much more serious part. Prophylaxis against epizootics is not the business of a small general staff of specialists but of the entire population of a country which must collaborate wholeheartedly and without exception. This is impossible unless each individual member is convinced that the measures taken will produce results. From the moment loopholes are inevitable, confidence is shaken and the whole structure totters even if it does not collapse. In other words, the over-simple argument the sanitary authorities come up against runs as follows: If they cannot prevent the outbreak of infection and the dissemination of the virus by certain produce, there is no point in all the rest of their measures and it would be vain to attempt to apply them. Certainly this reasoning is specious, but if one remembers the very real restrictions that the authorities impose on the economic life of the countryside during epizootics, one can and must understand that the circles affected always seek pretexts, even the most baseless, for avoiding the immediate expenses and losses prophylaxis always involves in the interests of an unquestionably real but more distant advantage.

The first question to be asked concerned the time the dry virus can be preserved on the various substrata that can become contaminated. The very thorough work done by the English committee and published in their second and third reports of 1927 and 1928 provided an answer of fundamental value. In 1954, during the 22nd session of the International Office of Epizootics, Traub and Moosbrugger presented two reports which, concise though they are, form a good resume of our present state of knowledge. Since these texts have been published, we shall refrain from repeating them, helpful though this would have been to us in our task, and instead endeavour to extract the salient facts.

What strikes one first of all is the differences between the periods of preservation, which in the laboratory vary between 2 days to 15 weeks. More important still, however, is the part played in these tests by the substratum. Hay, for example, is unquestionably preservative in action, even in the form of sterile extract. Bran has an identical effect in the presence of air but is a poor medium in the absence of oxygen. The virus also remains infectious for a long time on hairs.

This variable action can be explained in two ways. The first, which commands general support, is that the substratum exercises a protective effect by a mechanism which is still unknown but which, it is thought, may be approximated to that of the pH or that of the oxidation-reduction potential. The second, to which we are inclined, is that the virus assumes another molecular arrangement, in which it is much less infectious but remains resistant for a longer period. Let us recall first of all Pyl's observations on the now classic phenomenon of re-alkalinization. In vitro foot-and-mouth disease virus preserves its virulence several hours when brought rapidly from pH 7.6 to 3. Brought back to pH 7.6 it loses it at once, although it preserves its virulence if it is brought directly to pH 9.5. For this reason Pyl admits two forms of virus, one at pH 7.6 which may pass to pH 3 and pH 9.5 irreversibly, and the other which exists at these two pH and can pass from one to the other without injury. Without subscribing unreservedly to Pyl's original conception, for we believe that the phenomenon is more complex, we hold that foot-and-mouth disease virus should not be considered as a fixed entity but rather as a molecule constantly varying internally. Certainly, in order to study it, we must take, as it were, a photographic aspect of one of its qualities. In so doing, we shall neglect what we may call for convenience other components without knowing whether they really exist permanently or whether they are only transient. In order to make the idea clearer we will give an example, although well aware that our comparison is probably very incomplete. In a solution of a salt which is not completely dissociated it is possible to determine the degree of dissociation. At the moment one proceeds to do so, part of the dissociated molecules are recombining and an equal number of complete molecules are dissociating. Statistically speaking, measurement is possible although the solution is always different from what it was immediately before. On our scale there is balance, in other terms, as Ch. Eug. Guye puts it, the phenomenon is on the scale of the observer.

We have seen in our introduction that in certain cases the infectiousness of the virus tends towards the monomolecular phenomenon where all statistics are frustrated. Certainly we can apply what our experiments have taught us, but in so doing, we must remember that we are undertaking an audacious extrapolation, that is to say, apparently paradoxical results will only be the reflection of our preconceived ideas.

If we appear to have deviated from our subject, we shall now see the consequences of this digression. What in our view has so long caused failure to recognize the importance of the dissemination of foot-and-mouth disease by vegetable produce is the substantial discrepancy between the enormous number of possibilities of infection and the very small number of foci that can be attributed beyond all doubt to this mode of contamination. I will give you an example. The first time we found the virus in fodder, 700 tons of the same consignment had

been distributed in Switzerland over a considerable area. Two outbreaks, involving only 2 animals, were concerned. If the virus had simply been diluted, we should have witnessed a series of simultaneous appearances. If, on the other hand, what we were dealing with was a qualitative modification of the virus, the disease would become manifest only in very rare cases where the organisms were electively receptive to this form. In fact, we have found confirmation in the laboratory in the observation made for the first time of abortive aphthae which are incapable of developing and generalizing but which regain their primitive infectious character in passage. Last year Willems in Brussels confirmed the phenomenon with a virus taken from the marrow of bones from quarters of meat which had been chilled for several months. Thus, the virus of foot-and-mouth disease can take a form which is infectious only under certain conditions that are seldom met with in practice. All the same, practically the whole of the produce may contain the virus in this form without any manifestation in the field, for with the same sack we were able to repeat our experiments and obtain precisely the same results every time.

On the other hand, we must not assume that the danger is so small that it has no practical importance, for once the disease has broken out, it spreads, particularly in countries hitherto unaffected, with a rapidity and violence with which we are only too familiar. Let us remember that the 1951-52 invasion in Europe began in a single focus confirmed a few miles from Hanover, and there are many more examples that could be cited.

Thus, once the power of foot-and-mouth disease virus to preserve itself in a more or less dangerous form on vegetable produce had been recognized, and that was the work achieved by the English committee, the second stage consisted in demonstrating the reality of the fact under practical conditions. The experiment could be carried out only in a country which was free from foot-and-mouth disease that imported fodder and where the possible causes of an outbreak could be studied exhaustively. It was because Switzerland fulfilled these 3 conditions that we were in a position to complete the practical demonstration of the English research. We have already referred to our observations on the form of virus found. In the course of our research we have ascertained that the complex phosphate of colloidal calcium obtained by ion-exchange between sodium phosphate and calcium chloride has an adsorbant and at the same time exponential effect on foot-and-mouth disease virus, that is to say, at a pH value of 9, its infectiousness is increased by one to three powers of ten. This action is more marked on the guinea pigs than on bovines.

The number of our tests may appear small, since we have only found the virus 4 times on vegetable produce originating in practice, but it seems adequate to us in view of the severe criteria we have applied in order to give the tests a positive meaning. In fact it is only when the test animals have generalized and when the primary aphthae have

been rather weak or transient in order to eliminate all risk of contamination with our working strains that we have admitted an infection by the produce referred to. We have not attempted to multiply the observations but on the contrary to augment the strictness of the conditions imposed on each.

Finally we made a study of all the outbreaks in Switzerland from 1947 to 1954 which seemed attributable to the importation of vegetable produce. Here again we took into account only cases where the following conditions were all fulfilled at the same time:

a) there had been no foot-and-mouth disease in the surrounding district,
b) no other cause had been revealed or suspected,
c) the suspected agricultural produce had been used during the 10 days preceding the disease,
d) several animals had been affected at the same time,
e) foot-and-mouth disease was rife in the countries where the produce under consideration originated.

Under these rigorous conditions we have observed, during these 7 years, 11 primary foci of foot-and-mouth disease in Switzerland. During the same period double this number also has been attributed to the same cause but these cases have not been taken into account because one or other of the imposed conditions was not fulfilled. It is a well-known fact that the origin of an initial outbreak in a country free from the disease is always very difficult to determine. To postulate infection by fodder in an importing country like Switzerland too often provides an easy solution in an arduous investigation. That is why we have had to impose strict criteria, for here again we prefer a small number of well-founded observations to a large number of cases where one or two dubious instances make one sceptical as to the whole.

We have classified the produce into 4 groups, but the list is not a limiting one.

a) Fodder. This is unquestionably the most dangerous since it comes into direct contact with the receptive animals. Untreated fodder, such as hay as well as concentrates in powder form (bran, lucerne, etc.), may be dangerous. There is, moreover, a point that has not been entirely cleared up and it is one to which the suppliers attach importance. It is that of the contamination of the virus-carrying produce. It is certain in the big producing countries the stock-raising and the crop-growing districts are separate. Thus, it may be asked how the virus has been able to attach itself to the produce which would never have had the least chance of contact with it. In our view this is to underestimate the genium epidemicum of foot-and-mouth disease virus on the one hand and to shift the problem

on the other, for the same question may be asked in regard to animals infected in a country free from the disease. Here is an example: In winter at a farm in the Bernese Oberland cut off by snow and at a time when there had been no outbreak confirmed for 20 years in this district and none anywhere in Switzerland for a number of months, there was a flare-up of foot-and-mouth disease a week after the beasts had been fed with hay coming from a country where the disease was endemic. Certainly the certificates stated that the produce had been cut in a zone where there had been no reports of the disease for 6 months within a radius of 20 kilometres, the hay itself had certainly been cut 8 months before and its fermentation heat should have killed the virus. If the fodder is turned down as an explanation, it will be necessary either to find another solution to which the same objections may be raised, or to admit that the disease was transmitted without a carrier across two chains of mountains 13,000 feet high. We could cite many more examples. We believe that it is undoubtedly necessary to maintain a critical spirit, but in two senses, without wishing to attribute everything to the fodder, we must not deny a priori the possibility of its being implicated by an act of faith which might easily lead to intellectual dishonesty.

b) Litter. Litter, that is straw and peat, may play a part in Europe both on the national and international scale. It was on straw that we once found fairly active virus. The greatest danger comes from packing material used as litter. Without wishing to overestimate the value of sanitary certificates, they may be said to correspond to the truth in the vast majority of cases and provide, if not a guarantee, at least a strong presumption of harmlessness. Packing straw on the one hand comes from an unknown source and the chances that it has come from contaminated districts where it could be freely sold for this very reason must not be left out of account.

c) Vegetables. When we discovered the virus on salsify peelings--very much to our amazement it may be said--we will admit that people thought we were exaggerating somewhat. In point of fact, these scraps had been given to pigs in a canning factory without prior cooking in order not to destroy the vitamins. In another case foreign vegetables had been washed in a fountain just before beasts from a neighbouring farm drank water from the same fountain. Finally we have observed a focus caused by scraps from a railway dining car, likewise amongst pigs. Naturally, the vegetables on sale at a greengrocery do not constitute a serious danger, but the few cases we have referred to show conclusively the tortuous and at first sight unlikely paths the foot-and-mouth disease virus may follow in its dissemination.

d) Seed. This fourth and last group may be of much greater importance than might be suspected. Switzerland, for example, must import every year several thousand truckloads of seed potatoes and on several occasions foot-and-mouth disease has broken out during the few

days following handling. Here again the produce in question came from countries that were certainly infected to a greater or lesser degree, but where, we were assured, no contact was possible between the cattle and the growing districts. Since in our country it was not possible either for there to have been contact between infected animals (these being slaughtered ruthlessly immediately after the disease is confirmed) and the beasts at the centres where the disease had just broken out, the only conclusion we could draw was that an outbreak was in itself impossible, the sole objection being, of course, that one had in fact taken place.

3. Protective Measures

It might be concluded from that foregoing, as we have already intimated, that in the face of an infection so diabolically insidious, protective measures to prevent its introduction are doomed to certain failure in advance. That this is not the case, however, is shown by experience. Certainly so long as entire countries are subject to permanent infection there will be isolated outbreaks. Present-day methods of combating the disease enable us to quell the outbreak completely with more or less dispatch. On the other hand, where it is a matter of stopping repeated and multiple introductions, modern methods, without being inoperable, are far less effective. It is not always a question of preventing all contamination but at least of limiting the incidents to a number not beyond the technical capacity of the sanitary authorities to absorb and keep under control. Thus, according to the economic position of each country, we can put into operation three systems--admittedly of unequal value--which are nonetheless made imperative by circumstances.

a) Total prohibition. This is the first solution to spring to mind, the easiest way out of the difficulty. In the first place it can only be applied in countries which are entirely self-supporting and these are not numerous. Moreover history shows that no Great Wall of China has ever prevented invasions. The feeling of false security thus induced has as its corollary a progressive weakening in the effectiveness of the measures taken to combat the disease inside the country, and, like fortifications, it saps the fighting spirit and paves the way to disaster. It must not be inferred that we think that a few outbreaks now and then would be a good thing, any more than we would advise the fire brigade to start a fire every year in order to keep in training. The country applying total prohibition must know that such a policy is not foolproof and keep its means of defence in a better state of preparedness than a state whose own measures, constantly tested in action, are corrected, improved and perfected by the pressure of events and in order to close the breaches that experience has revealed.

b) Differentiated prohibition. This is the system that is most commonly practised, the most usual, the most general. It could in

itself be made the object of a paper longer than the present one. We must content ourselves at best with extracting the general principles. In the first place imported produce should come from regions that have been entirely free from foot-and-mouth disease for a sufficiently long period. We can see straight away that this statement contains two indeterminate quantities, one relating to area and the other to time. Both are unavoidable and the international conferences that have tried to give them a more precise character have had to give up the idea, leaving the importing country to make its own decision according to circumstances. And quite rightly so, for how, to take an example, can tiny Switzerland, which has been an organized state for centuries and where every infected beast is slaughtered, be compared in regard to the exportation of cattle with Brazil with its vast expanses--some still virgin--where the infection is permanent. For this reason the system reduces itself to a comparative study of the economic necessities which render the importation of produce unavoidable and the risks which it is possible to accept. Finding the equation for these 3 variables will determine the solution. For example, after a prolonged drought the mass importation of fodder will have to be accepted even at the cost of outbreaks of foot-and-mouth disease, which may be anticipated and the appropriate measures taken.

Then again, the more efficient the sanitary authorities of the exporting country, the more applicable the system becomes. Far be it from us to sermonize certain countries and to make comparisons which in our view are out of place, for our generation is not responsible for the historical and economic evolution of which it is the beneficiary and we have no grounds for congratulating ourselves on work done by our predecessors. It may be said, nevertheless, as a matter of actual fact, that the struggle against epizootics is easier and more effective in certain countries, and this should be an encouragement to others, for it shows that solutions which at first sight seem beyond the bounds of practicability have in fact been achieved, and obstacles which appear unsurmountable have been well and truly surmounted. International collaboration worthy of the name demands a genuine and unfailing effort from each and not merely a division into those that give and those that receive.

c) Disinfection. Finally the third system, if it may so be called, consists simply in an effort to destroy the virus before it comes into contact with susceptible organisms. It is an attempt to interrupt the cycle of contagion at one particular point only, and we have said that for foot-and-mouth disease this method is doomed to failure. Although an inert object may be sterilized with comparative ease, the process is not practicable on the scale of the world economy. Moreover there exists at present no way of disinfecting agricultural produce without taking the nature out of it, with the few exceptions referred to. Plant produce in particular cannot be effectively treated before its arrival at its final destination. Only there and only in certain cases (meals, fodder, vegetables) will thorough cooking have some chance of success. But unprepared fodder, litter, and seed cannot be treated with the means at our disposal at present and constitute a latent danger which, small though it may be, is nonetheless formidably real.

Conclusion

In the dissemination of foot-and-mouth disease all agricultural produce will constitute a danger varying widely in seriousness but never altogether absent so long as foot-and-mouth disease continues to be rife in many parts of the globe. Preventive methods with one or two exceptions are only comparatively effective and must be supplemented by the methods of eradication which have proved their worth and which should be applied, encouraged and developed by every means at our disposal for the benefit of all and sundry.

EPIZOOTIOLOGY OF VESICULAR EXANTHEMA OF SWINE

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INTRODUCTION

This is an important period of time in modern veterinary medicine for our Department of Agriculture and the Nation's livestock industry. It is a period of many events associated with animal disease work--events such as the dedication of this great new modern animal disease laboratory on Plum Island and Congressional approval and appropriations for the construction of another new modern animal infectious disease laboratory at Ames, Iowa; events concerning animal disease research, the development and expanded use of diagnostic and biologics testing procedures, and new and expanded work in disease control and eradication programs. It seems, therefore, most fitting and proper that the dedication of this laboratory shall also serve as a milestone, marking our progress and entrance into a new era of progressive modern veterinary science for a more healthy and disease-free livestock industry.

It was seriously hoped that the dedication of this new laboratory could have served as the milestone marker for the termination of vesicular exanthema eradication program and the extinction of that virus from the face of the earth. Vesicular exanthema had not appeared clinically in swine outside of California for 18 months, nor in the State of California for 9 months, until August 6, 1956, when it was again diagnosed in New Jersey.

HISTORY

The history of the virus disease, which causes vesicular exanthema of swine, is one of the most mysterious and intriguing stories of modern medicine. To men of science it could be placed in the category of one of those stories which are stranger than fiction. The origin of the causative agent for this disease is still unknown. There is no scientific proof nor even sound evidence that this disease has ever occurred naturally anywhere in the world other than in the United States.

Vesicular exanthema was enzootic for only California for 20 years, 1932 to 1952, during which time practically all of the California experimental work was done by Traum and his junior colleagues, Madin, Bankowski, and their associates. Their recent publications give an excellent summary covering the history for that period (8, 2).[1/]

[1/] Figures in parentheses refer to references at end of this article.

The disease was probably first observed in swine alone by man on April 23, 1932, 24 years ago, on a ranch near Buena Park, Orange County, California. It spread only to swine on other ranches in Orange County and the adjacent counties of Los Angeles and San Bernardino. It was diagnosed as foot-and-mouth disease and eradicated by slaughter and burial of all infected and exposed animals, followed by cleaning and disinfection of premises (9). Cattle, horses, and guinea pig inoculation tests were negative (14).

About 1 year later, in March 1933, the disease appeared about 100 miles from the 1932 outbreak, in swine only, in San Diego County, California. Swine, cattle, horses, and guinea pigs were again inoculated. The swine and some inoculated horses were positive, but all cattle and guinea pigs were negative. The disease again was diagnosed as foot-and-mouth disease and eradicated by slaughter. Even though an official diagnosis of foot-and-mouth disease was made for the 1932 and 1933 outbreaks, it has been reported by Traum and coworkers that the diagnoses were not made without reservations, as they at that time believed they were dealing with a virus other than the virus of foot-and-mouth disease.

Following the 1933 outbreak, Traum (14) reported a new vesicular disease of swine, as a result of his experimental studies, and suggested the name "vesicular exanthema." His conclusion was later confirmed by other workers in the United States (5) and Europe (13), and the virus was proved to be immunologically different from the foot-and-mouth diseases and vesicular stomatitis viruses.

A third large outbreak of the disease occurred 15 months later in 1934, about 500 miles from San Diego, near San Jose, California, in the San Francisco area, and later appeared again in the Los Angeles area. This time, slaughter and indemnity payments were discontinued and disease control was attempted by a rigid quarantine and disinfection program. Outbreaks in California occurred again in 1935 and 1936; then the disease was not observed for a 42-month period from June 1936 to December 1939.

An outbreak occurred in San Mateo County, California, in December 1939, and within 6 months involved one-fourth of the State's swine population. Since that time the disease has occurred annually in California until December 1955, when infection from the last outbreaks, which occurred in November of that year, was eradicated.

In June 1952, the disease was identified in swine at Grand Island, Nebraska, and was traced to a shipment of raw-garbage-fed swine from Cheyenne, Wyoming, that probably were infected from garbage from a transcontinental train coming from California. It spread to 18 States within 6 weeks and by September 1953 had appeared in 40 States and the District of Columbia (11). The Secretary of Agriculture, on August 1, 1952, declared a state of emergency, and an eradication program was started.

Control and eradication is carried out cooperatively by State and Federal regulatory officials, each State developing its program within its borders and the Federal program regulating interstate control. The principal difference in this national vesicular exanthema eradication program from previous vesicular disease eradication programs is the addition of a prohibition against feeding raw garbage and of controlling the marketing of garbage-fed swine. As of September 1953, 46 States, all except New Jersey and Connecticut, had enacted legislation requiring the cooking of garbage to be fed to swine. As a result of this national vesicular exanthema eradication effort, there were only 15 new disease outbreaks during 1955, the last outbreak in that year outside of California occurred in February 1955. There had not been an outbreak of vesicular exanthema in California for 9 months, and there had not been an outbreak outside of California for 18 months, when the previously mentioned outbreak of August 6, 1956, occurred on a single farm in New Jersey.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE ETIOLOGICAL AGENT

The vesicular exanthema causative agent is a filtrable virus, the particle size of which falls in between that of foot-and-mouth disease virus, which is smaller, and vesicular stomatitis virus, which is larger. The virus is capable of passing through Gradacol membranes of 44 mu average pore diameter and is calculated to be 13 to 20 mu in size (4, 7).

The thermal death point for the virus varies in accordance with virus concentration, heat inactivation time, and the nature of the vehicle surrounding the virus. The minimum heat-treatment requirement for inactivation of 10-percent centrifuged virus saline suspensions was 64°C for 15 minutes, 62°C for 30 minutes, and 60°C for 60 minutes.

Information on the effect of a range of pH variations on the virus is inadequate and too limited to pinpoint the endpoints on either the acid or alkaline side. On the acid side, 0.5% hydrochloric acid, pH 2.7, mixed with virus for 15 minutes resulted in virus inactivation, whereas 0.25% hydrochloric acid, pH 4.3, for 15 minutes did not inactivate 5 percent virus. On the alkaline side, the results were as follows: 1.0% NaOH, pH 12.8, for 1 minute caused inactivation of 5 percent virus; 0.5% NaOH, pH 12.3, for 15 minutes caused inactivation of 5 percent virus; 0.05% NaOH, pH 10.73, for 24 hours did not inactivate 10 percent virus; 2.0% Na_2CO_3, pH 10.89, for 1 minute did not inactivate 10 percent virus; and 2.0% Na_2CO_3, pH 10.89, for 15 minutes caused inactivation of 10 percent virus. These results indicate the vesicular exanthema virus may be more sensitive to alkaline pH than the vesicular stomatitis virus.

Ultra-violet irradiation for 10 minutes caused inactivation of 10 percent virus. The virus was contained in a flat dish. The virus film depth was 0.15 cm, but the dish was slowly agitated during the period of irradiation with a shaking machine. Two G30TA clear Westinghouse sterilamps, transmitting 3,050 angstrom units each, were mounted 6 inches above the virus suspension.

IMMUNITY

There appears to be a difference in the immunity pattern conferred by vesicular exanthema as compared with foot-and-mouth disease or vesicular stomatitis. The immunity in swine convalescent to B-51-type virus is more solid and of longer duration than that observed from foot-and-mouth disease or vesicular stomatitis in animal species susceptible to those viruses. Insufficient information for comparison, however, is available on swine immunity from all three diseases. The 50-percent duration of immunity from B-51-type vesicular exanthema virus was about 20 months in two separate experiments.

There has been practically no difference observed between the degree of tissue immunity and humeral immunity in vesicular exanthema-convalescent swine. In contrast to foot-and-mouth disease, vesicular exanthema-recovered animals, if immune, are solidly immune and are negative to intradermal virus challenge. Generalizations usually occur if an animal has no tissue immunity at the epithelial tissue inoculation site.

Immunity frequently develops in vesicular exanthema experimental swine without evidence of clinical infection. Nothing is known about the duration of this type immunity.

IMMUNOLOGICAL VIRUS TYPES

There probably is not another virus of animals which has a more intriguing multiplicity of plurality-type pattern than the one being unraveled for vesicular exanthema of swine during its relative short life span of 24 years. No one will ever know the complete immunological type variations which have occurred.

The 1932-outbreak virus which had been collected was destroyed by governmental orders along with the infected and exposed animals. A series of virus collections made during 1933 and 1934 were forwarded to the then Bureau of Animal Industry, Animal Disease Station, at Bethesda, Maryland, where Dr. A. B. Crawford (5) identified four immunologically distinct types. He named them A, B, C, and D. It has been reported that type A was collected in 1933 and the other three types were collected during the 1934 outbreak. Additional type plurality information probably could have been developed at an earlier date had Crawford continued his studies, but the Government ordered the work stopped and the removal of all virus to minimize the danger of disease outbreaks in the Eastern States. Unfortunately, the California workers also lost their early virus collections.

During vesicular exanthema outbreaks in California in 1940-42, a second attempt was made by the California workers to collect field viruses and establish a repository of virus types. Three immunologically distinct types were recovered and identified as 1940-A, B, and C, but were also subsequently lost.

A third series of vesicular exanthema virus type collection was started in 1948 and has continued up to the present time. These presently accumulated types are now identified alphabetically in the order of isolation, followed by the year collected. The identity of presently known types are as follows: A-48, isolated by Madin and Traum (7), and B-51, C-52, D-53, E-54, F-55 (previously reported as type 330), and G-55 (previously reported as type 332), isolated and identified by Bankowski et al.(2). It should be clearly understood there is no known immunological relation between the alphabetic identification of types used for the 1934, 1940, and 1948 isolation series, with the exception of the 1934-B, which will be discussed later. In addition to the present seven types, it is quite probable there are three or four additional existing types.

Some of the early vesicular exanthema virus isolations were forwarded to the British Foot-and-Mouth Research Institute at Pirbright, England. Brooksby (4) found two viable virus specimens among the stored samples. One was a 1934-B from the first series of isolated types identified by Crawford, and the other was a 1943-101 strain, which had not previously been identified in any of the above-mentioned three series of virus isolations. The 3 available types at that time of the third isolation series, A-48, B-51, and C-52, were forwarded to Brooksby for comparative studies, and he reported these 5 strains to be distinct antigenic types. This does not eliminate the possibility of 1934-B and 1943-101 strains' being identical with D-53, E-54, F-55, or G-55, so the present number of known existing types is between 7 and 9.

At the present time the California laboratory has one virus strain and the Beltsville, Maryland, laboratory has another virus strain, both of which are showing irregular immunological characteristics. Future studies will determine if these are new types.

THEORIES OF VESICULAR EXANTHEMA VIRUS ORIGIN AND VIRUS MUTATIONS

I have previously stated in my paper (page 74) that "the origin of the causative agent for this disease is still unknown. There is no scientific proof nor even sound evidence that this disease has ever occurred naturally anywhere in the world other than in the United States." There has been a lot of oral discussion without practically any published information on the subject. Three plausible theories of virus origin have been advanced. There probably are others. These theories are as follows:

(1) The disease may have been introduced with virus-infected garbage from foreign ships. It could have washed ashore from ocean-going vessels and later could have been picked up by swine along the California beaches. In this connection it must be assumed the disease did or does exist in some foreign countries. It is also of interest that since the 1929 outbreak of foot-and-mouth disease in California, a

regulation has been in effect forbidding ships to bring garbage into her ports. Another matter of theoretical speculation is whether there was one or probably three separate entries of virus, because all infected and exposed animals were destroyed in the 1932 and 1933 outbreaks. Duckworth (6, pp. 12-13) stated, concerning the 1934 outbreak, "it is inconceivable that infective material of any kind could have carried over from either of the two earlier outbreaks and found its way into a swine herd 500 miles distant, 15 to 26 months later."

(2) Another theory concerning more the ancestory of the virus rather than the method of entry is that perhaps this vesicular exanthema virus is a descendant of some of the so-called foot-and-mouth disease virus strains which, according to different early European workers (13), would affect swine but not cattle.

(3) The third theory is that this virus may be a mutant from some other virus, which would logically be either foot-and-mouth disease or vesicular stomatitis. Foot-and-mouth disease did occur in California during 1924-25 and again in 1929. Whether vesicular stomatitis occurred in California before 1932 is not known, but the New Jersey type of the vesicular stomatitis virus was recovered and identified from California outbreaks during the early 1940's.

The foot-and-mouth disease outbreak in California during 1924-25 spread to deer and possibly other forms of wildlife. Attempts were made to destroy the deer in order to control the disease, but it was reported large numbers of deer were never killed. The foot-and-mouth disease virus could have become adapted and mutated in deer or some other wildlife, followed by infestation of swine.

The important and interesting fact concerning the possible vesicular exanthema virus mutation hypothesis is that there is more and more field evidence giving support to the mutation theory. Crawford's 1934 typing results gave the earliest clue to possible rapid virus mutation, when 4 immunologically different types were found, 3 types of which occurred within a single outbreak area.

Different research workers and control officials have been suspicious of possible virus mutation because of recurrences of infection in the same herds in California. Research workers, however, have not been successful in producing evidence of virus mutants experimentally. Bankowski et al.(2) have presented the best evidence of virus mutation through their attention to virus typing. During 3 years and 8 months, from October 1951 to June 1955, they successfully typed 227 samples of 325 samples received from 126 outbreaks in California swine and 199 import swine shipments, which were infected on arrival in California. Only type B-51 was recovered from all 139 of the 199 import samples, which was the same type that occurred outside of California; there was no evidence of mutation there.

Virus mutation is strongly suggestive from the typing of the 88 samples from the 126 native California outbreaks, because there was a new immunological type identified for each successive year, B-51, C-52, D-53, E-54, F-55, and G-55, and more important it appears the old types are disappearing. A-48 was not found at all during this time, and the still older types, 1934-B and 1943-101, have probably disappeared, as they were shown by Brooksby (4) to be different from A-48, B-51, and C-52, which were the only virus types identified in California during the 5-year period 1948 to 1952, inclusive.

The Animal Disease Station vesicular disease virologists at Beltsville, Maryland, have immunologically typed vesicular exanthema virus from outbreaks in 15 States--Arkansas, Florida, Illinois, Maryland, Massachusetts, Mississippi, Missouri, Nebraska, New Jersey, North Carolina, Ohio, South Carolina, Texas, Virginia, and Washington--and the District of Columbia during 1952-1954.

Particular attention was given to the typing of virus from many States because we expected this vesicular exanthema virus to start mutating. All virus samples typed out B-51, which is the type virus that occurred in the initial 1952 Grand Island, Nebraska, outbreak.

On August 6, 1956, 18 months after the last vesicular exanthema outbreak in the United States outside of California, which occurred in South Carolina in February 1955, there was a new vesicular exanthema outbreak in New Jersey. This outbreak developed 24 months after the last appearance of the disease on that farm in August 1954 and 22 months after the last vesicular exanthema outbreak in New Jersey, which occurred in October 1954 on the adjoining hog farm (15). This New Jersey type virus appears to be a type other than B-51, as type B-51, 4-month immune swine were susceptible to virus challenge. One may conclude from these observations that we probably now have a B-51 mutant virus. The New Jersey infected herd of swine was slaughtered August 9, and cleaning and disinfection of premises were completed on August 17.

We believe the future experimental work on virus genetic and mutation studies with vesicular exanthema virus will be much more productive than in the past because of the development of new working tools. The complement-fixation test based on 50-percent hemolysis appears to be of value in serotype studies, and the successful adaptation of this virus to tissue culture is opening up many new possibilities.

ROUTES OF VIRUS INFECTION AND ELIMINATION

Whether all immunological types have similar infectivity characteristics is not known, but the author and associates (10) have demonstrated with type B-51 that the intradermal route of inoculation is the most sensitive to vesicular exanthema virus exposure. It requires 10 to 100 intradermal snout minimum infectious doses to make one intravenous or subcutaneous minimum infectious dose and 100 to 1,000 intradermal

snout minimum infectious doses to make one feeding minimum infectious dose. This information would seem to indicate the most natural route of exposure to be by epithelial tissue contact; however, additional experiments have shown the most common natural route of exposure is more probably by way of oral ingestion. For example, spleen tissue containing two intradermal minimum infectious doses per gram of tissue gave negative intradermal results because 2 to 3 cc of spleen saline suspension is about the largest quantity which could be successfully inoculated intradermally into the pig snout, whereas 500 grams of the same spleen produced lesions after feeding.

Virus elimination from intravenously exposed swine, measured by contact exposure with groups of susceptible test pigs, started 12 hours before vesiculation and ended 84-108 hours after vesiculation. The period of viremia closely approximates the time of viral elimination. Virus elimination has been demonstrated in saliva and feces before vesiculation occurred. Urine samples were negative for virus. Virus survived for only 72 hours in infected pens when tests were made with susceptible pigs.

RESERVOIRS, CARRIERS, AND INAPPARENT VIRUS INFECTIONS

Reservoirs--One of the most distinguishing characteristics of vesicular exanthema virus is its apparent inability to naturally infect any species of animal other than swine. Some successful artificial transmissions have been reported in horses (5, 14), hamsters (7), guinea pigs (7), and dogs (3). However, these transmissions could not be propagated successfully beyond one to six serial passages and then only with certain serotypes of the virus. Negative transmission trials have been reported for mature cattle, calves, sheep, goats, mice, rats, rabbits, hedgehogs, chicken embryos (7), and adult chickens (12). Human cases of infection have not been observed nor reported. The narrow virus-susceptibility host range does not eliminate the possibility of virus reservoirs in some forms of wildlife, rodents, arthropods, helminths, or cold-blooded animals, but it does suggest a decrease in such possibilities.

Carriers--The term "carrier," as used by most veterinarians, refers to an animal which was infected with a disease and recovered clinically but following recovery continued to carry the infectious agent in some part of its body, from which it may later eliminate infection and cause exposure of susceptible animals. At Beltsville, we have given considerable attention to the possibilities of carrier infections in our work with B-51 type vesicular exanthema virus. We have regularly placed susceptible swine under observation with convalescent swine which previously had vesicular lesions. At the end of 30 days' exposure, these test pigs were removed for challenge to determine whether or not immunity had developed and a new group of susceptible test pigs were added to the convalescent swine. There has not been a single test pig which developed infection or immunity in these carrier experiments. As a result of these studies, we now have developed a different philosophy in our

thinking about carriers. We do not want to leave the impression that carriers are not possible, but we think the potential danger of carrier animals in swine which have had vesicular lesions and therefore would have high antibody serum titers are practically insignificant in comparison with swine from the same infected herds which did not develop clinical lesions.

Inapparent or nonclinical vesicular exanthema--Some people, from a practical viewpoint, probably classified these inapparent forms of vesicular exanthema as carriers. It is considered improper to include these animals as true carriers because they are, in our opinion, infected with active multiplying virus and a very dangerous group of animals which should be differentiated from the recovered clinical cases. It is our theory that inapparent or a smoldering form of vesicular exanthema continues its propagation in a herd among the animals which did not have vesicles and therefore do not have antibodies for protection against infection. Groups of susceptible swine added to the herd from time to time supply the fuel for the fire which keeps the virus alive so the disease could survive for months or even years in large feeding establishments without being observed.

We have evidence that such a state of inapparent infection frequently does occur from challenge of lesionless experimental animals that were immune. Bankowski (1) has reported evidence of such inapparent infection in an infected herd which was quarantined for 90 days, during which time it was inspected biweekly without detection of clinical lesions. At the termination of the 90-day period, the quarantine was released for immediate slaughter. Tissues from these swine when fed to susceptible test pigs produced lesions in 7 of 10 pigs. The recent August 6 outbreak of vesicular exanthema in New Jersey is, we believe, another result from inapparent vesicular exanthema, according to Animal Disease Eradication Branch reports (15). The farm where the outbreaks occurred never has been entirely free of swine since the last outbreak of vesicular exanthema, which occurred there in August 1954. In working with this virus we made eleven rapid serial passages before we felt safe in making inoculation into type immunes and other species. The vesicular lesions produced were very small, many sites of inoculation were entirely negative. Some inoculated pigs were entirely negative, and at the fifth passage we thought for a time we had lost the virus passage. Such a virus could still be propagating itself without detection in the States where the total swine population was not removed following a vesicular exanthema outbreak.

The cooking of garbage, if continued, should prevent the introduction of this virus into other garbage-feeding establishments.

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EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

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The European Commission for the Control of Foot-and-Mouth Disease was instituted with the object of creating close collaboration among western European and, if possible, all European countries and thus of bringing the diseases under better control in Europe than in the past. The Commission was set up in 1954, the immediate impetus for its creation being the extensive epizootic throughout Europe in 1951 and 1952.

Continental Europe has several times been swept by foot-and-mouth disease and in most countries there was little possibility of self-defense. When the Schmidt-Waldmann vaccine appeared in 1938, it was felt that at last an effective weapon had been created against the disease and there was a flood of optimism. To a great extent I was one of these optimists and my optimism had some historical foundations. It will be remembered that the first experiments in vaccinating against foot-and-mouth disease were carried out by the French scientists Vallee, Carree, and Rinjard in 1925 and 1926 with a virus inactivated by formalin. It will also be remembered that the first experiments in vaccinating with a virus adsorbed to aluminium hydroxide were proposed by the Danish scientist Sven Schmidt.

Shortly after entering the Danish Virus Institute on the Island of Lindholm in 1935, I had the exciting experience of seeing these experiments being carried to an end. The results were so highly satisfactory that this new adsorbate vaccine opened up considerable possibilities for the effective control of foot-and-mouth disease.

During the early years after the introduction of the vaccine, there was considerable reason for optimism. The results of vaccination in Germany and in Denmark were so satisfactory that the new vaccine could be considered as one of the best in existence. The opinion became widespread that with the help of this vaccine the disease could be kept almost completely under control. Unfortunately, this optimism was not borne out by the results during the 1951-52 epizootic.

International collaboration in the control of epizootics took a concrete form in 1924 when the International Office of Epizootics was formed in Paris. This organization is worldwide and is greatly appreciated for its valuable advisory activities. Over the years OIE

has studied problems concerning foot-and-mouth disease at many meetings and passed many useful resolutions. It was, however, felt by a number of European countries that foot-and-mouth disease was of sufficient importance to warrant a separate organization, dealing specifically with foot-and-mouth disease problems in Europe. At a conference convened by the Food and Agriculture Organization of the United Nations in 1952 in Copenhagen it was proposed that a European Commission for the Control of Foot-and-Mouth Disease should be created and this proposal was supported by the representatives of most of the European countries present at the meeting. After the epizootic had abated, interest in such a commission waned to a certain extent, and when the commission was eventually set up in June 1954 only 6 out of 20 European countries became members, namely Denmark, Ireland, the Netherlands, Norway, the United Kingdom, and Yugoslavia. Since then a further five countries have adhered to the Commission, namely Austria, Iceland, Italy, Portugal, and Turkey. There is a well-founded hope that most of the remaining western European countries will eventually become members.

The European Commission for the Control of Foot-and-Mouth Disease has its seat and secretariat at the FAO headquarters, Rome, and as far as the administration is concerned the secretariat is responsible to the Director-General of FAO. The technical activities of the Commission are directed by the Commission through its Executive Committee.

On adhering to the Commission a country undertakes to put into operation certain quarantine and sanitary measures for the control of foot-and-mouth disease and also one or more of the following policies: A slaughter policy, slaughter together with vaccination, maintenance of a totally immune cattle population by vaccination, and vaccination in zones surrounding outbreaks.

Member countries undertake to collaborate with and assist other member countries in all joint measures for the control of foot-and-mouth disease, and in particular in supplying vaccine and virus where necessary.

Members also undertake to make such arrangements for the typing of virus from outbreaks of foot-and-mouth disease as may be required by the Commission, and to report immediately outbreaks of foot-and-mouth disease in their countries.

As far as the functions of the Commission are concerned, the following should be mentioned. The Commission is to ensure that all members are provided with technical advice regarding any problems connected with the control of foot-and-mouth disease; that comprehensive information about outbreaks of foot-and-mouth disease and identification of virus is collected and reported as quickly as possible; that special research work which may be considered necessary is carried out; that information with regard to national programs for the control of and research into foot-and-mouth disease is collected.

Since its formation, the European Commission has, in particular, worked with the following objectives: To elaborate an effective reporting system; to appoint an international laboratory for the typing of strains of virus; to encourage special research work; and to visit European countries for the purpose of obtaining information on national plans for the control of foot-and-mouth disease.

In connection with the last widespread epizootic criticism was voiced that the inter-European reporting system in use had not worked satisfactorily. The Commission considered that a good reporting system was of vital importance and discussions with the director of OIE were, therefore, initiated last year in order to elaborate such a system. It was agreed urgently to recommend the veterinary authorities of the different countries to report immediately to OIE the following information regarding foot-and-mouth disease:

When an outbreak is confirmed in a country free from the disease or when, in a country in which the disease is already present, the epizootic begins to spread, the information should be given at once by telegram. The telegram should contain essential information about the outbreak. In addition, another telegram should be sent as soon as the virus type has been confirmed.

The information received in the telegram should be followed by a letter containing additional information and stating the measures taken.

OIE undertakes to forward to all countries directly interested the particulars received by telegram and to send copies of all information received to the secretariat of the Commission in Rome.

When the new reporting system is working satisfactorily, it should be possible to ensure that all the necessary information becomes available without delay. The secretariat of the Commission discusses the reporting system with the veterinary authorities of the different countries and endeavors to ensure the smooth operation of the system.

For a long time the need has been felt for a central reference laboratory for the classification of virus types and variants. On the Commission's initiative a seminar, convened by the Organisation for European Economic Cooperation and arranged by the Government of the Netherlands, was held at the end of last year in Amsterdam to discuss the typing and cultivation of the viruses of foot-and-mouth disease. In the conclusions of the seminar, it was strongly recommended that storage and classification of all virus strains and the preparation of anti-sera should be entrusted to the Virus Research Institute at Pirbright, United Kingdom.

The Commission considers that its interest in research work must be an important part of its task. Various projects have been discussed

during the different meetings of the Commission. At its second session in March 1955 it was urgently recommended that the two following research projects should be carried out in the very near future: The holding of a seminar on the typing and cultivation of foot-and-mouth disease viruses; and experiments to determine the duration of immunity in cattle vaccinated with culture virus vaccine.

The seminar was held in Amsterdam last year. One of the recommendations was that a group of technical workers should be formed and should meet at least once a year in different laboratories: the object being to establish close collaboration among research workers on foot-and-mouth disease.

The duration of immunity after vaccination with culture virus vaccine is an important question on which insufficient particulars are at present available. The Commission is now taking the necessary steps to obtain all available information on the subject and to initiate controlled experiments.

A further project under discussion is the possibility of blocking the virus in already infected herds. It will be remembered that Waldmann recommended in 1938 that vaccine should not be used in already infected herds because of the negative phase following its application. However, for many years it has been common practice in Italy to vaccinate in already infected herds, with apparently satisfactory results. During the 1951-52 epizootic an attempt was also made to vaccinate infected herds in Belgium and Denmark, also with quite good results. If it proves possible to block the virus, the idea would be to slaughter infected animals and then to protect the normal animals by blocking the virus, killing free virus by disinfection. So far very little research has been done along these lines. Perhaps it might be a possible project for study by the new institute which we have just seen opened.

An important part of the work of the Commission is, of course, close contact with European countries. With this aim in view, members of the secretariat have, during the past 2 years, visited many of the countries in western Europe.

The foot-and-mouth disease situation in Europe has been relatively calm since the 1951-52 epizootic and at the present time only three countries, Belgium, France, and Italy, still have a considerable diffusion of the disease. These countries are centrally situated and constitute a lesser or greater threat to other European countries. Italy is a member of the Commission and the secretariat made an early start on the study of the situation in that country. The disease is of greatest importance in the north of the country in the Po Valley where there is a great concentration of animals and people and where large cattle markets are held and there is an intensive traffic of both men and livestock. From this area the disease spreads southwards over the rest of the country, especially

through trade in livestock. The chance of the disease spreading to other countries from Italy is limited since Italy does not normally export cattle. On the contrary, the country imports extensively from other European countries, especially into the Po Valley area, and it is thus that epizootics of foot-and-mouth disease have been introduced. Although it might seem that the control of foot-and-mouth disease in Italy is more or less a domestic problem, it must also be viewed from an international standpoint.

France is centrally situated, bordering many other European countries. A permanently infected France is, therefore, a threat to all neighbouring countries. In any plan for the eradication of foot-and-mouth disease from Europe, France has to be considered as the cornerstone.

In Belgium the majority of cattle have been vaccinated and are well protected against foot-and-mouth disease. Systematic vaccination, however, has not yet been carried out and among the unvaccinated animals a considerable amount of foot-and-mouth disease still appears. During the past few months spreading of the disease from Belgium has taken place into the Netherlands, Germany, and Switzerland.

Before the last war it used to be a maxim that important European epizootics were caused by invasion from outside, from Russia, Asia Minor, or Africa. It was also believed that foot-and-mouth disease epizootics started as type O virus infections and were followed by A virus infections, and that C outbreaks occurred only sporadically. One should never make maxims about foot-and-mouth disease. Our present knowledge shows that European epizootics can arise from any source of infection and can start by A as well as by O infections and that grave epizootics can also be caused by C virus.

The first task in Europe must be to eradicate foot-and-mouth disease from Belgium, France, and Italy. An example of how the disease can suddenly flare up is shown by the recent Swiss epizootic. Belgian pigs in transit to Italy caused an almost explosive epizootic, with about 100 outbreaks. Once again, however, the Swiss system of combating foot-and-mouth disease, namely a combination of slaughter, vaccination, and strict sanitary measures, has proved its efficacy.

The question of eradicating foot-and-mouth disease in Belgium, France, and Italy cannot be considered as being only strictly national problems. It is of great interest to the whole of Europe and all European countries ought to take part in such a campaign. How a campaign should be carried out will, of course, depend on the situation in each country and part of the country. The Swiss experience will be most valuable, as will be that gained in Mexico. In some parts of Europe the most satisfactory method of dealing with the disease will be to carry out slaughter of infected animals and to practise systematic vaccination with suitable vaccines within areas or even throughout the whole country, together with the necessary restriction on movement and disinfection.

The FAO proposed the formation of a European Commission for the Control of Foot-and-Mouth Disease because it was felt that a regional approach to the problem would be the best solution, leading eventually to the institution of a world-wide campaign. In Europe the campaign must be supported to the utmost by the three international organisations particularly interested in animal disease control, the OIE, the OEEC, and the FAO. There is every reason to be optimistic and to believe that the disease will be eradicated within a limited number of years. European facilities for the production of vaccine are much better than they were a few years ago and our scientific knowledge of the disease is also much greater. Once the disease is eradicated from Europe it should not be too difficult to prevent its entrance.

ANIMAL DISEASE RESEARCH AT PLUM ISLAND

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INTRODUCTION

Research on foot-and-mouth disease has interested the United States Department of Agriculture for many years. During the 1924 outbreaks in California and Texas the need for more technical information on the disease was recognized. The Department therefore established a Commission that carried on research in Europe in 1925 and 1926. At that time experimentation with the virus of foot-and-mouth disease was prohibited in the United States because no safe and suitable facilities were available for the purpose.

In 1947, following the outbreak in Mexico, the Department's Foot-and-Mouth Disease Advisory Committee recommended that further research be initiated at once, in cooperation with the European laboratories, to assist the Mexico-United States Commission for Eradication of Foot-and-Mouth Disease and to strengthen protection against the disease in the United States. The cooperative research started in 1948 in Denmark at the State Veterinary Research Institute for Virus Research, in England at the Research Institute at Pirbright, and in the Netherlands at the State Veterinary Research Institute has been since continued. This program has aided the progress of research in these countries and has afforded valuable training and experience for United States personnel, some of whom are now on the Plum Island staff.

The Committee and the Department also recommended that research facilities be developed in the United States as soon as possible. In 1948 Congress authorized (Public Law 496, 80th Cong.) establishment of a special laboratory for the purpose. The law required that the laboratory and related facilities for the research be established on a coastal island. Research was authorized on foot-and-mouth disease and such other animal diseases as, in the opinion of the Secretary of Agriculture, might constitute a threat to the livestock industry of the country.

Following the outbreak of foot-and-mouth disease in Canada in 1952, Congress appropriated 10 million dollars for building a laboratory. Plum Island, the site of the U. S. Army's Fort Terry, was selected as the location. The entire island was transferred to the Department of Agriculture in 1954. Limited research was started in existing facilities that had been rehabilitated and adapted for safe experimentation. A new, more complete laboratory is now almost complete. The

early availability of a facility for research, together with such auxiliary facilities as an administrative building, cafeteria, motor and other shop areas, emergency power station, and other structures permitted initiation of research about 2 years before this would otherwise have been possible.

Future research at the Plum Island Animal Disease Laboratory will be devoted primarily to investigations of foot-and-mouth disease; but, if need arises, attention may be given to diagnosis and research on other foreign plagues. Research at the laboratory includes investigation of diagnostic procedures, modes of transmission, susceptibility of various species, breeds and classes of animals, virus propagation and destruction, and other phases important to a more complete knowledge of preventive measures, including immunization procedures and fundamental studies of the virus. The laboratory is also responsible for the conduct of laboratory tests required for specific diagnosis of foot-and-mouth disease and other diseases as directed.

STUDIES OF VESICULAR STOMATITIS

The research program started on Plum Island in June 1954 and continued for about 1 year with the virus of vesicular stomatitis. This virus was chosen as a simulant of the foot-and-mouth disease virus during the first phases of the program for the purpose of training of personnel in techniques applicable to vesicular diseases in general, testing of the laboratory facilities, development of appropriate safety procedures, and accumulation of reference stocks of virus and antiserum of the 2 types of vesicular stomatitis. Work was directed along 2 main lines: First, establishment in the United States of a safe laboratory and the capability for application of critical techniques for differential diagnosis of the vesicular diseases. Second, exploration of virus propagation techniques.

Familiarization with diagnostic techniques was established promptly and has been maintained for the past 2 years. Early success was achieved in propagating virus in guinea pig kidney explants and trypsinized guinea pig kidney cells. The investigations were confined to one type of the virus at a time. The New Jersey type was passed serially through more than 50 successive passages, the Indiana type, more than 15 passages. Sample fluids collected from such cultures at various times regularly produced the typical disease in all known susceptible species of animals that were tested.

Cytopathogenic effects of vesicular stomatitis virus were first observed in outgrowths of bovine tongue epithelium. Six-day old cultures of such tissue evidenced cytopathogenic effects within 24 hours after inoculation with the virus. Degeneration of the cell outgrowths was observed in such cultures within 3 days after exposure

to the virus. The cytopathogenic effect was characterized by pyknosis, resulting in granular, nearly spherical, contracted cell casts in which nuclei and cytoplasm became indistinguishable, the outgrowths appearing lacelike.

The same effects were next observed in outgrowths of explants of guinea pig kidney. In experiments with trypsinized guinea pig kidney cell cultures bathed in nutrient fluid in tubes the pathogenic effects on the cells were clearly observable microscopically.

In addition to the production and assay techniques for vesicular stomatitis virus in tissue cultures, as just described, attention was paid to the production of vesicular stomatitis virus in suspensions of surviving guinea pig and bovine kidney cells. The work with surviving kidney cells has precedent in the work of Frenkel and Van Waveren who proved the feasibility of use of suspensions of other types of tissue for promoting the growth of vaccinia virus nearly 20 years ago. More recently, Frenkel, using tongue epithelium, employed the suspension method in the production of foot-and-mouth disease virus for use in compounding vaccine in large quantities.

Several tissue culture and suspension methods of producing vesicular stomatitis virus have been used at this station. Virus titers demonstrated in such cultures have reached 10^7 ID_{50} per ml in explants of bovine tongue epithelium, explants of guinea pig kidney, outgrowths of trypsinized guinea pig and bovine kidney cells, and trypsinized guinea pig and bovine kidney cells in suspensions. The highest virus concentrations in cultures and suspensions were most frequently recorded at 24 and 72 hours following infection. Using this technique the volume was increased from 2 to 200 ml without decreasing the virus yields per ml. After reaching peak virus concentrations at 24 hours with the culture technique and 72 hours with the cell suspension method, the degree of infectivity fell off rapidly upon further incubation.

The choice of host for determination of the concentration of an infectious agent is important because of differences in their sensitivity to infection. For purposes of comparison both New Jersey and Indiana types of vesicular stomatitis virus were titrated in the tongues of calves and adult bovines, embryonating chicken eggs, Swiss albino mice, guinea pigs, the tongues of chickens, and tissue cultures of guinea pig kidney cells. Three- to four-week old mice inoculated intracerebrally and embryonating chicken eggs inoculated on the chorioallantoic membrane were found to be the most sensitive hosts and gave high LD_{50} values. The ID_{50} values in tongue-inoculated adult cattle and tissue cultures were approximately equal and next in order of sensitivity. Titrations on calf and chicken tongues gave lower ID_{50} values than any of the preceding hosts. The guinea pig gave the lowest values of all obtained in the comparative series.

Studies have been made with vesicular stomatitis virus, types New Jersey and Indiana, to determine the temperature at which such virus was rendered noninfective by heat, and the utility of such heat-treated material as antigen in the complement-fixation test. Infected bovine tongue epithelium was the material used for these studies. Supernates of centrifugated 10-percent virus suspensions were heated for 30 minutes in 30 ml flat-bottomed round vials containing small bar magnets that were fitted with one-hole rubber stoppers through which thermometers were inserted. This assembly was placed in a precision water bath resting on a large magnetic stirrer which kept the bath and tubed suspensions stirred during the time of heating.

The infectivity of virus suspensions treated in this manner and immediately chilled at the end of heating was tested in suckling mice (8 to 9 days old) inoculated intra-abdominally, cattle inoculated intradermalingually, guinea pigs inoculated in the metatarsal pads, embryonating chicken eggs (7 to 9 days), and bovine kidney cultures. Under these conditions, heating at temperatures of 56° or 60° C for 30 minutes uniformly rendered suspensions of vesicular stomatitis-infected bovine epithelium noninfective for all media in which they were tested.

The same heated virus suspensions were used as antigen in the complement-fixation test. It was found that the degree of fixation obtained with the heated antigen was only slightly less than with unheated controls. There was only a two-fold lesser titer of heated versus unheated vesicular stomatitis virus suspensions. It was concluded that vesicular stomatitis virus in suspensions of bovine tongue epithelium treated as described may be used in the complement-fixation test in open areas without exposing personnel, normal animals, or clean laboratory areas to infection or contamination.

Human infections with vesicular stomatitis virus, based on serological findings, have been reviewed by Hanson. Similar observations have been made at the laboratories of the Animal Disease and Parasite Research Branch at Beltsville, Md. In the course of investigations of vesicular stomatitis virus at Plum Island, a professional worker was exposed to the New Jersey type of vesicular stomatitis while inoculating cattle in the tongue or examining infected cattle. Twenty-four hours after this exposure the man experienced malaise and general depression as well as fever. A blood sample taken 48 hours after exposure and inoculated into embryonating chicken eggs produced death of the embryos by the third day after inoculation. Bacteriologically sterile material from these embryos were serially passaged four times. Material from the fourth passage had an LD_{50} titer of 10^{-9} in embryonating chicken eggs.

Neutralization of the agent recovered from this person was demonstrated in inoculated chicken eggs with two samples of serum taken from the worker 14 and 80 days after exposure. The agent after the fourth passage was neutralized by bovine vesicular stomatitis antiserum of the New Jersey type but not by normal bovine serum nor earlier samples of

serum taken from the worker. Intraplantar inoculations in guinea pigs demonstrated neutralization of the recovered agent by New Jersey type bovine antiserum but not by normal bovine serum. Complement-fixation tests using the recovered agent as antigen in the presence of the New Jersey and Indiana types of hyperimmune guinea pig serum were positive for the New Jersey type only. Although there is considerable clinical and serological evidence of the presence of vesicular stomatitis virus infections in man, this constitutes the first proved instance of vesicular stomatitis viremia in man.

A serological method described by workers at Plum Island for the identification and titration of vesicular stomatitis virus, involves the use of virus, immune serum, fresh horse serum, heat-inactivated bovine serum, and guinea pig erythrocytes. The results obtained using this technique have been shown to be specific. This method has not been suggested as a substitute for the standard complement-fixation test, but it promises to be at least a useful supplemental test in diagnosing vesicular stomatitis. It may be applicable to antigen-antibody systems which fail either to fix guinea pig complement or in which there is anticomplementary action.

STUDIES OF FOOT-AND-MOUTH DISEASE

In July 1955, studies of foot-and-mouth disease were started in the same laboratory building that had been used for vesicular stomatitis virus investigations, after the building had been cleaned and disinfected. Samples of several types of the virus were brought to this station from the Research Institute at Pirbright, England. In compliance with United States law, these materials were transported by ocean-going vessel and transferred offshore to a smaller craft stationed at Plum Island. To date work has been confined to type A virus, Pirbright strain 119.

In applying the tissue culture techniques used with vesicular stomatitis virus to foot-and-mouth disease virus, we found that some modifications would be required to obtain high concentrations of the foot-and-mouth disease virus. These alterations were several; however, the two changes that seemed to effect the greatest improvement were the substitution of bovine kidney cells for guinea pig kidney cells and the modification of the nutrient fluids used in such cultures. In addition to propagation of the virus of foot-and-mouth disease in guinea pig and bovine kidney cells, it has also been grown in cultures prepared from kidney cells of sheep and swine and in cultures of mouse tumor cells. Because bovine kidneys are easily available and relatively large in size and the cytopathogenic lesions produced by foot-and-mouth disease virus in bovine kidney cultures are clearly delineated, cultures prepared from bovine kidneys have been preferred over others in our tissue-culture work.

The foot-and-mouth disease tissue culture and chemical work, which has been in progress for the past year, will be reviewed in four phases as follows:

The first phase--cultures in tubes have been used for assays of foot-and-mouth disease virus and its neutralizing antibodies. Briefly, trypsin-dispersed bovine kidney cells in nutrient fluid are placed in 0.4 ml quantities in culture tubes. The tubes are incubated at 37°C for 3 days at which time they receive a change of fluid. Later the tissue cultures in such tubes become confluent with cellular outgrowths of mixed epithelial and fibrocytic cells and are suitable for use in virus or antibody titrations. In such titrations, cytopathogenic lesions may be seen within 3 hours of infection but final readings for calculation of TC ID_{50} values are made at the end of 48 hours. A scale-up of the culture tube method has been accomplished in Roux flasks. Such flasks containing large confluent layers of cells and 75 ml of nutrient fluid have been used for the routine production of foot-and-mouth disease virus and for some 70 serial passages of the virus in tissue culture.

The second phase--the work involved the production of virus in suspensions of bovine kidney cells. This technique differs from the culture method in that the virus is incorporated directly into suspensions of trypsin-dispersed bovine kidney tissue. As much as 12 liters of fluid containing 10^7 TC ID_{50} per ml of virus have been produced in a single container by this means. Titrations of virus produced in this manner have been carried out by both TC ID_{50} and plaque assays. It has been found that the peak of infectivity is reached in 12 hours by this method in contrast to 72 hours in the case of vesicular stomatitis virus. It is believed that this method offers considerable promise as a means of producing virus in large quantities for vaccine, chemical, and other studies.

The third phase--the Dulbecco plaque technique for the assay of viruses in monolayer tissue cultures has been modified to serve as a relatively simple and highly accurate method for the assay of Type A foot-and-mouth disease virus. Bovine kidney cultures are grown in flat-bottomed 100 mm Petri plates in the presence of media of the same composition as that used in the production of tube cultures, that is, 97.5% Hanks salt solution, 2% bovine serum, and 0.5% lactalbumin enzyme hydrolyzate. The plates are incubated in cans sealed with plastic tape. After the confluent cell cultures have been inoculated and overlaid with the agar-nutrient-fluid mixture, they are incubated separately in sealed plastic bags. The need for gassing with CO_2 is thus eliminated. The infected plates are examined after 48 hours and 72 hours and in scattered light the virus plaques appear as holes in an otherwise uniform outgrowth of cells. Each plaque is considered as arising from a single infective unit of the virus which may be a single particle or a combination of particles, probably the former. A count of the plaques thus indicates the number of infectious units in the original inoculum.

These studies have shown that the accuracy of the method is dependent upon strict control of a number of variables, including the volume of

inoculum, the time and temperature for absorption of the inoculum before the overlaying mixture is applied, and the composition, pH and volume, of the overlay. When these variables have been properly controlled, the plaque assay method has a high degree of precision, reliability, and reproducibility. Approximately 95 percent of the time counts of populations in the range of 47 to 66 vary from the mean by less than 31 percent, as compared to the tube-assay method using the 50 percent endpoint, in which the results are reproducible in 95 percent of the cases only within ± 350 percent of the mean titer.

The fourth phase--the tissue culture and biochemical work involved the use of the tissue-culture virus production and assay techniques in obtaining fundamental knowledge about the production of virus and its stability to pH, temperature, and formaldehyde. From these studies much information has been developed. It is interesting to note that the latent period before the appearance of virus progeny is only 2 hours long, that a peak titer of $10^{8.6}$ plaque forming units per ml is reached at 11.5 hours after which the titer decreases at a rate predictable from the known thermal inactivation of tissue culture foot-and-mouth disease virus, type A. The average yield of plaque-forming units per cell was 370 in this experiment. Curves just as precise as this have been developed for the stability of tissue culture foot-and-mouth disease virus at different hydrogen ion concentration, various temperatures and in the presence of formaldehyde, but time does not permit their inclusion in the present review.

The tissue-culture techniques have also permitted us to make important advances toward purification and ultimate isolation and identification of the physical particle which carried the infectious principle of foot-and-mouth disease. Two apparently different kinds of spherical particles in the size range of the smallest known animal viruses are now under suspect but the final determination of which of the two, if either, is foot-and-mouth disease virus awaits further experimental proof.

The action of gaseous ethylene oxide on foot-and-mouth disease virus (A119) in infected bovine tongue epithelium has been investigated to determine the efficacy of this substance for the sterilization of potentially contaminated objects such as photographic film, microscopes, and other materials which, because of their nature, will not withstand ordinary steam sterilization.

The ethylene oxide used in these experiments had been prepared in pressurized cans containing liquid ethylene oxide dissolved in liquid Freon 12. This mixture was released into an autoclave from which the air had been evacuated to 25 inches of Hg. In three trials centrifugated 10-percent suspensions of infected bovine tongue epithelium were exposed in a thin layer in Petri dishes for 4 hours.

Following this, the tissue suspension was recovered from the Petri dishes, diluted and inoculated intra-abdominally into suckling mice, trypsinized bovine kidney cell cultures, and the tongues of adult cattle. None of these developed evidence of foot-and-mouth disease infection, whereas control suspensions left at room temperature for the same time were fully infective for the same animals and medium. The complement-fixing antigen in infected tongue tissue was unaffected by exposure to ethylene oxide for 4 hours.

Experiments were conducted to determine the comparative susceptibility of three age groups of cattle, 2, 6, and 18 months, respectively, from the same breeder of grade Hereford cattle, to type A foot-and-mouth disease virus strain 119, when exposed by contact or intradermalingual inoculation. Dilutions of virus were inoculated into four animals of each of the three age groups in 4 separate trials. The ID_{50} endpoints were calculated and comparisons were made.

Because of space limitations in the contact experiments, it was not possible to mix the three groups; however, five animals each of the 2- and 18-month groups were placed in one room at the same time. One animal of each of these two ages was inoculated and permitted to mingle intimately with the remaining eight uninoculated animals. The following week five animals each of 6 months and 18 months age were placed together, one animal of each age being inoculated and placed in the same room in intimate contact with the other eight animals. In the contact exposure experiments determination of infection in the respective groups was based on the time of rise in temperature and the time of development of lesions in the uninoculated animals. From the results of these comparative titrations and contact exposures it was concluded that there was essentially no difference in the susceptibility of 2, 6, and 18 months old animals to type A, Strain 119 foot-and-mouth disease virus.

During the past year there was an opportunity to obtain 20 steers consisting of four animals in each of five groups of different line-bred Hereford stags approximately 2 years of age. These animals had been on feeding experiments since a young age to compare weight gains of the progeny of the 5 lines of breeding. The animals were made available by the Animal and Poultry Husbandry Branch, Agricultural Research Service. These animals were brought to Plum Island and inoculated intradermalingually to determine their susceptibility to the virus of foot-and-mouth disease, in comparison with the standard experimental cattle used here. The so-called standard cattle, grade Hereford steers, aged 16 to 20 months, are obtained from a contract producer who maintains a closed herd in which random breeding by purebred bulls is practiced.

A comparative titration conducted in the six groups of cattle showed essentially no difference in the susceptibility of the 5 separate groups of line-bred cattle and the Plum Island "standard" cattle.

Based upon published information, it has been accepted generally that heating of foot-and-mouth disease virus at 56° to 60°C for 30 minutes is sufficient to destroy its infectivity. Generally, those who have conducted experiments of this type have used guinea pig virus, and the infectivity or innocuity of heated preparations has been determined through inoculating guinea pigs. Some few workers have heated bovine virus preparations but mostly have employed guinea pigs in tests of virus inactivation. The work done prior to the early 1920's was, of course, carried out in larger susceptible animals.

Safety precautions desired in laboratories at Plum Island have necessitated a search for methods of producing noninfectious antigens satisfactory for use in the complement-fixation test. One method that was tried involved the use of heat in an attempt to destroy infectivity of foot-and-mouth disease virus in suspensions of bovine tongue epithelium while maintaining complement-fixing activity. Considerable effort has been devoted to development of a controllable system for heating virus preparations for use in such tests.

In the same assembly used for heat inactivation of vesicular stomatitis virus the supernates of 10-percent suspensions of foot-and-mouth disease virus were heated at temperatures ranging from 56° to 80°C for periods of time ranging from 30 minutes to 24 hours. All heated virus suspensions were inoculated in dilutions 10^{-1} to 10^{-4} intradermalingually in cattle, intraperitoneally in mice, intradermically in the metatarsal pads of guinea pigs and into cultures prepared from trypsin-dispersed bovine kidney cells. Unheated portions of the same preparations were inoculated similarly for control purposes and to determine the 50 percent endpoints of infectivity of the same unheated materials.

In this work it has been shown that 10-percent suspensions of infected bovine tongue epithelium must be heated for 6 hours at 80°C to entirely destroy infectivity for the bovine when inoculated intradermalingually in 0.5 ml amounts of dilutions 10^{-1} through 10^{-4}. When 50 ml quantities of virus suspensions heated at this temperature and time were inoculated by the intramuscular or subcutaneous routes, however, there was evidence of vestigial active virus. One of four cattle inoculated intramuscularly and one of four inoculated subcutaneously developed lesions of foot-and-mouth disease on the 8th and 10th day post-inoculation. The other eight animals inoculated intravenously with 50 ml of the heated suspension or intradermalingually with 10 ml remained negative. The results obtained by inoculation of such materials into mice, guinea pigs, and tissue culture were seldom in full agreement with those obtained by inoculating cattle; in fact, guinea pigs and mice were rarely infected, indicating that final observations of the infectivity of foot-and-mouth disease suspensions must be made in the bovine when the greatest sensitivity is desired.

The results of a large number of controlled tests of heated virus of bovine origin (epithelium) strongly indicate the inadequacy of any

except unexpectedly high temperatures and long-continued exposure in completely eliminating infectivity of foot-and-mouth disease virus. Some preparations heated for as long as 4 hours at 80°C have been found infective for cattle, and in a few instances infection developed, after extended incubation periods of 8 to 10 days, in cattle injected intramuscularly or intradermalingually with massive doses of virus heated as long as 6 hours at that temperature. With preparations heated at levels above 65°C for periods extended beyond an hour and inoculated in 0.1 ml quantities at several sites on the tongue in the course of routine titrations, the incubation period also tended to increase progressively as either temperature or time of exposure were increased.

In contrast with cattle, guinea pigs and mice rarely developed infection after inoculation of the usual doses of any dilution of materials heated at 60° or higher levels for 30 minutes. In only about one-third of the cases did tissue culture results approximate those obtained in cattle. Tissue cultures were, however, considerably more susceptible to inoculations of heated virus than either guinea pigs or mice.

During these trials, there were instances of absence of infection in cattle injected intradermalingually with low dilutions of heated virus, while the same preparation in higher dilutions produced the disease. This may be attributed to greater quantities of inactivated virus in the lower dilutions tending to mask or interfere with minimal or vestigial infective virus, a hypothetical interference phenomenon exhibited by some other viruses such as influenza. This manifestation, however, has not been regular with all preparations in cattle and it has not been observed in inoculated tissue cultures, mice or guinea pigs.

The apparently high degree of resistance of foot-and-mouth disease virus to heat that has been observed is so opposite to previously reported experiences as to cause serious concern. It has been observed, however, that the American cattle used at Plum Island generally have exhibited susceptibility to one or two higher tenfold dilutions of virus than has been reported by other observers using virus of the same strain and origin in different cattle under possibly different conditions in other countries. Results to date clearly suggest a hypersusceptibility in our cattle, that is possibly attributable to the complete absence of exposure of any animals in the country for so many years. They would thus be expected to develop infection as a result of minimal exposure.

Ignoring the fact of possible species adaptation, guinea pigs are generally conceded to be less susceptible than cattle to the same quantity of virus. Experiences at Plum Island give little support for use of guinea pigs in detection of minimal quantities of virus. The apparently similar relative refractivity of mice in these experiments, however, is surprising.

In preliminary trials bovine epithelial virus heated for as long as 24 hours at 56°C retained its complement-fixing antigenicity, but at the same time was still infective to a degree for cattle. In contrast heating at 70°C for 30 minutes destroyed its antigenic properties.

In tissue cultures incubated at 37.5°C beyond the peak of virus concentration, approximately 90 percent of the virus apparently is lost within 24 hours. This deterioration appears to proceed as a first order reaction with a corresponding reduction on succeeding days so that within 5 or 6 days virtually no active virus remains. The time of complete loss of virus activity, however, has not been determined.

FOWL PLAGUE

In addition to the above-described investigations, we have been assigned the task of producing fowl plague antiserum to be made available to certain designated laboratories which a Departmental Committee on Exotic Diseases has selected as diagnostic centers. We have available for immediate shipment a quantity of fowl plague antiserum prepared from a Brescia strain. In another month Alexandrian strain antisera will be ready. We will also have N-virus antisera available soon. The Brescia antisera has been safety tested, lyophilized in 3 ml quantities; it was prepared by hyperimmunizing adult chickens with three inoculations of formalinized fowl plague virus, followed by the administration of live virus.

It has been a pleasure to participate in this, the first symposium of the Plum Island Animal Disease Laboratory, particularly with the group who have made such fine presentations during the past 2 days. I hope it has been possible for all of you to become acquainted with Plum Island staff members whose work has been reviewed here today and to discuss scientific matters of common interest. I trust that the papers presented will stimulate free discussion and questioning. Questions concerning the work just summarized will be handled by members of the Plum Island staff.

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